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ORIGINAL

Nigella sativa extract attenuates benzene induced oxidative DNA damage and abnormality in hematological parameters in rats

El extracto de Nigella sativa atenúa el daño oxidativo del ADN inducido por el benceno y la anomalía en los parámetros hematológicos en ratas

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Abstract

Background: Benzene exposure harms the genetic material and hematopoietic system. This effect appears in the form of excess 8-hydroxy-2-deoxyguanosine (8-OHdG) plasma levels and abnormalities in peripheral blood cells' count. Nigella sativa showed antioxidant properties that may overcome benzene exposure side effects. This study aimed to evaluate the potential protective effect of N. sativa against benzene-induced DNA damage and hepatotoxicity in rats.
Methods: The study was conducted on 26 Wistar rats. They were divided into three groups; group (A) eight rats (30,8%) were administered 100 mg benzene/ kg body wt, 5d/week, group (B) nine rats (34.6%) were administered (100 mg/kg BW/day) benzene plus (100 mg/kg BW/day) Nigella sativa extract via gastric gavage for 4 weeks, and nine normal rats (34.6%) without any chemical agents designated as a control group. Plasma 8-OHdG levels and peripheral blood count were used to evaluate Nigella sativa protective effect in the face of benzene exposure.
Results: A significant increase of 8-OHDG plasma levels among benzene-exposed rats compared to the control group (p = .000). Nigella sativa and benzene administered group showed a significant decreased 8-OHDG plasma levels as compared to benzene exposed animals. There was a significant improvement of RBC count, hemoglobin concentration, WBC count, and platelet count (P = .003, .000, .000; .000 respectively) in N. sativa and benzene administered group when compared to benzene exposed rats..
Conclusions: The current study provided evidence that the DNA damage and the hematotoxic effect of benzene can be counteracted by Nigella sativa.

Keywords: Benzene, Nigella Sativa, 8-OHdG, blood cells, antioxidant, hematotoxicity, genotoxicity.

Resumen

Antecedentes: La exposición al benceno tiene un efecto perjudicial sobre el material genético y el sistema hematopoyético. Este efecto se manifiesta en forma de un exceso de niveles plasmáticos de 8-hidroxi-2-deoxiguanosina (8-OHdG) y de anomalías en el recuento de células sanguíneas periféricas. La Nigella sativa demostró tener propiedades antioxidantes que pueden superar los efectos secundarios de la exposición al benceno. El objetivo de este estudio es evaluar el potencial efecto protector de la N. sativa contra el daño al ADN y la hematotoxicidad inducidos por el benceno en ratas.
Métodos: El estudio se realizó en 26 ratas Wistar. Se dividieron en tres grupos: el grupo (A), en el que se administró a ocho ratas (30,8%) 100 mg de benceno/kg de peso corporal, 5 días/semana; el grupo (B), en el que se administró a nueve ratas (34,6%) (100 mg/kg de peso corporal/día) benceno más (100 mg/kg de peso corporal/día) extracto de Nigella sativa por vía gástrica durante 4 semanas, y nueve ratas normales (34,6%) sin ningún agente químico, designadas como grupo de control. Se utilizaron los niveles de 8-OHdG en plasma y el recuento de sangre periférica para evaluar el efecto protector de Nigella sativa frente a la exposición al benceno.
Resultados: Hubo un aumento significativo de los niveles plasmáticos de 8-OHDG entre las ratas expuestas al benceno en comparación con el grupo de control (p = .000). El grupo administrado con Nigella sativa y benceno mostró una disminución significativa de los niveles plasmáticos de 8-OHDG en comparación con los animales expuestos al benceno. Hubo una mejora significativa del recuento de glóbulos rojos, la concentración de hemoglobina, el recuento de glóbulos blancos y el recuento de plaquetas (p = 0,003, 0,000, 0,000; 0,000 respectivamente) en el grupo administrado con N. sativa y benceno en comparación con las ratas expuestas al benceno.
Conclusiones: El presente estudio aporta pruebas de que el daño al ADN y el efecto hematotóxico del benceno pueden ser contrarrestados por Nigella sativa.

Palabras clave: Benceno, Nigella Sativa, 8-OHdG, células sanguíneas, antioxidante, hematotoxicidad, genotoxicidad.

Introduction

Benzene, a hydrocarbon chemical compound derived from crude oil and gasoline, is broadly used as a solvent or intermediate for the synthesis of industrial chemicals such as plastics, polymers and detergents and is also a component of petroleum products[1,2]. It is considered an environmental pollutant and known for its hazardous effect against human health, especially when administered at substantial levels[3,4]. It is widely used in our daily life activities. It can be administered through cigarette smoking, combusting and evaporation of gasoline, and automobile emissions, which make its exposure unavoidable and poses a serious risk to both industry workers' health as general population[5,6]. Certainly, there is much evidence indicating the direct link between chronic exposure to high concentration of benzene and suppression of bone marrow, development of aplastic anemia, and various hematological malignancies[3,7,8]. Other studies have also reported the cytoreduction of blood cells in workers exposed to benzene's low concentration, indicating that benzene can exert hepatotoxic effects even at the lower permissible exposure limit[9,10].

The harmful effect of benzene on blood cells is recognized over 30 years, yet the mechanisms by which benzene induce toxicity are not clear[11]. The metabolism of benzene is most likely responsible for its cytotoxic effect by introducing an oxidative stress. Human body can eliminate 50% of the absorbed quantity of benzene through inhalation, while a low quantity of benzene accumulates in lipid tissues due to its solubility in lipid, and the rest is metabolized mainly in the liver through the cytochrome P4502E1 (CYP2E1) systems[12,13]. The resultant metabolites, including benzene oxide, catechol, and hydroquinone 2, can form redox cycling and lead to the generation of reactive oxygen species (ROS)[13]. These ROS can induce direct oxidative damage to the cellular components such as nucleic acid, proteins, and lipids and may also act through signaling molecules[14]. In normal conditions, the accumulation of ROS in the human body is regulated by antioxidant defense systems through certain metabolizing and scavengers to counteract the ROS oxidative damage[15]. However, both the increase in ROS production and insufficient protection by antioxidant factors result in inadequate protection from oxidative stress, which allows for an increase in DNA damage and subsequent genomic instability if the damage overwhelmed DNA repair capacity mechanisms[15]. Various studies have utilized different biomarkers, such as 8-hydroxy-2-deoxy Guanosine (8-OHdG), for evaluating the oxidative DNA damage[16].

Recently, there is an interest in utilizing plant-derived natural products as a natural antioxidant source with a beneficial effect on general health and different diseases, including cancer[17]. *Nigella sativa* L. (*N. sativa*), also called black seed, is historically used as a remedy with versatile medicinal applications[18]. *N. sativa* contains various compounds, such as thymoquinone (TQ), volatile oil, fixed oil, proteins, carbohydrates, minerals, vitamins, saponins, and alkaloids, which are essential for different biological properties of the seed[19]. Several studies have shown wide biological and pharmacological properties of *N. sativa* including, antioxidant, anti-inflammatory, antidiabetic, anti-hypertensive, anti-tumor and anti-microbial effects[20-24]. The antioxidant activity of *N. sativa* has been well documented in the literature and shown through studies that demonstrated the ability of *N. sativa* extracts to protect blood or organ tissues from oxidative damage induced by various chemicals such as alpha-lipoic acid, Streptozotocin, in animal models[25-27]. Given the recognized benzene-induced oxidative hematotoxicity and DNA damage linked with hematological malignancies, a search for protective agents against such toxic efafects is required. *N. sativa* is an attractive strategy in this context. The current study aimed to assess the potential protective effect of *N. sativa* against benzene-induced DNA damage and hepatotoxicity in rats.

Materials and methods

Chemicals

N. Sativa extract and benzene were purchased from Sigma Aldrich, St. Louis, USA, and an 8-hydroxy-2-deoxy Guanosine (8-OHdG) assay kit were purchased from Cayman's Chemical Co. (USA). All the reagents used in this study were of analytical grade.

Animal and experimental protocol

Male Wistar rats with 200-250g body weight from the animal house of Najran University, Najran, Saudi Arabia were used in this study. All experimental works were conducted according to the National Institutes of Health guidelines for the use, handling of laboratory animals (NIH Publications No. 8023, revised 1978). All protocols and experiments related to animal work were approved by the Institutional Animal Use and Care Committee in Najran University.

A total of 26 rats were employed in the study. The animals were divided into three groups; group (A) of eight rats (30.8%) were administered 100 mg benzene (Sigma Aldrich, > 99.8% purity)/ kg body wt, 5d/ week for 4 weeks. Benzene was administered in 1 ml of corn oil via gastric gavage. Group A designated as Benzene exposed. Group B of nine rats (34.6%) were administered (100 mg/kg BW/day) benzene plus (100 mg/kg BW/day) Nigella sativa extract via gastric gavage for 4 weeks. Group B is denoted as NIGL+Benzene. Group (C) of nine normal rats (34.6%) without any chemical additive served as a control group. Group C designated as control. Blood samples were collected via cardiac puncture post-anesthesia with light ether. About two ml of blood was collected in two EDTA-containing

sterile tubes; Blood samples were processed for plasma detection of 8-OHdG and complete blood count.

Blood picture

Hematological parameters were studied using an automated Blood Cell Counter (COULTER® LH 500 Hematology Analyzer - Beckman Coulter, USA).

Determination of 8-ohdg levels

8-OHdG plasma levels were measured using Cayman's 8-hydroxy-2-deoxy guanosine assay kit purchased from Cayman's Chemical Co. (USA). This assay detects both free 8-OHdG and DNA-incorporated 8-OHdG. This assay principle is based on the competition between 8-OHdG and 8-OHdG acetylcholinesterase (AChE) conjugate (8- OHdG Tracer) for a limited amount of 8-OHdG monoclonal antibody.

Statistical analysis

The 8-OHdG plasma levels and hematological parameters were analyzed statistically using the SPSS software (IBM SPSS Inc., version 20, Chicago, Illinois, USA), and results were expressed as mean values. The statistical measurements for the differences in plasma mean 8-OhdG levels and mean values of hematological parameters between studied groups were based on student *t*-test. Significant difference is denoted by *P*-value < 0.05 and highly significant if the P-value < 0.001.

Figure 1: Evaluation of the difference in plasma concentration of 8-OhdG in studied groups. The plasma concentration of 8-OhdG in the benzene-exposed rats was significantly higher when compared with the control group ($p < 0.05$). NIGL+ benzene group showed a significantly decreased plasma concentration of 8-OhdG than the benzene exposed group only ($p < 0.05$). NIGL denotes *N. Sativa.*

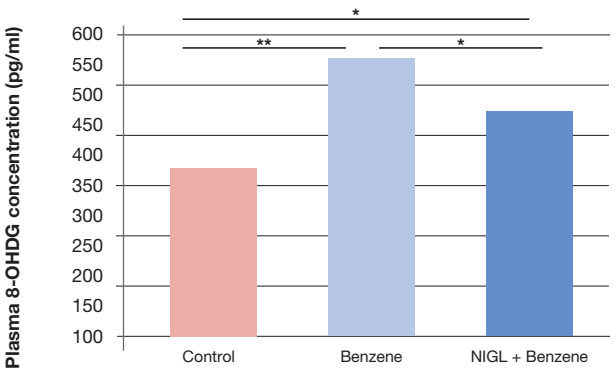

Table I: Significance of changes in plasma concentration of 8-OhdG.

Groups	Mean value + Standard Deviation	*t- test*	*p value*
1) Control VS. Benzene		8.286	0**
Control Benzene	383.78 + 57.779 564.75 + 22.676		
2) Control VS. NIGL+Benzene		3.436	0.004**
Control NIGL+Benz	383.78 + 57.779 480.00 + 44.721		
2) Benzene VS. NIGL+Benzene		4.661	0.001**
Benzene NIGL+Benzene	564.75 + 22.676 480.00 + 44.721		

**p-value is significant at the 0.01 level.
*p-value is significant at the 0.05 level. NIGL denote *N. Sativa*

Results

Evaluation of the oxidative stress response

Plasma 8-OhdG was measured between animal groups to detect the oxidative DNA damage induced by benzene and the potential protective effect of *N. sativa* against benzene-induced DNA damage. The first analysis focused on comparing the difference in plasma concentration of 8-OhdG between group A (Benzene exposed) and Group C (Control). The analysis revealed a significant increase of mean plasma concentration of 8-OHDG in benzene exposed rats in comparison to the control rat group (*p value* = .000), indicating that benzene resulted in oxidative stress, which recognized by an increase in the concentration level of 8-OHDG in animals exposed to benzene (**Figure 1**, **Table I**). Interestingly, Group B (NIGL+Benzene) showed a significantly decreased mean concentration level of 8-OHDG as compared to group A (benzene exposed animals) (p-value = .001). In contrast, the mean level of 8-OHDG in Group B (NIGL+Benzene) is significantly higher than that in group C (Control Group), indicating that co-administration of *N. sativa* extract along with benzene may result in scavenging the oxidative stress generated by benzene (**Figure 1**, **Table I**).

Evaluation of hematological parameters

The hematological parameters were also measured in all groups and results were compared with control groups.

The exposure to benzene showed a noticeable effect on blood cell as depicted by the changes in RBC count, Hb concentration, RBC indices, WBC count and platelet count between Group A (Benzene exposed) and group C (Control) (**Figure 2**, **Table II**). Certainly, there was a significant decrease in WBC count (p-value = .000) and platelet count ((p-value = .000) in Group A (Benzene exposed) as compared to group C (Control). Intriguingly, Group B (NIGL+Benzene) showed no statistical difference in RBC count, Hb and RBC indices compared to Group C (control rats). However, there was significant decrease in WBC count and significant increase in platelet counts between Group B (NIGL+Benzene) and Group C (control rats) (*P*-value = .000, *P*-value = .000, respectively) (**Figure 2**, **Table II**). Furthermore, a comparison of hematological parameters between Group A (Benzene exposed) and Group B (NIGL+Benzene) revealed a pronounced difference in all hematological parameters except MCV, MCH values. There was significant decrease in RBC count, hemoglobin concentration and WBC count (*P* value= .003, *P* value= .000, *P*-value = .000; respectively) and significant increase in platelet count (*P* value= .000) between Group A (Benzene exposed) and Group B (NIGL+Benzene). These results indicate the influence of N. sativa extract in reducing the hematotoxicity induced by benzene in rats.

Figure 2: Comparison of hematological parameters between studied groups. A) RBC Count, B) Hb Concentration, C) Mean Corpuscular Volume (MCV), D) Mean Hemoglobin Concentration (MCH), E) Platelet Count and F) WBC count. N.S indicates non-significant (p≥0.05), * indicates p<0.05 and ** indicates p<0.01. NIGL denote *N. Sativa*.

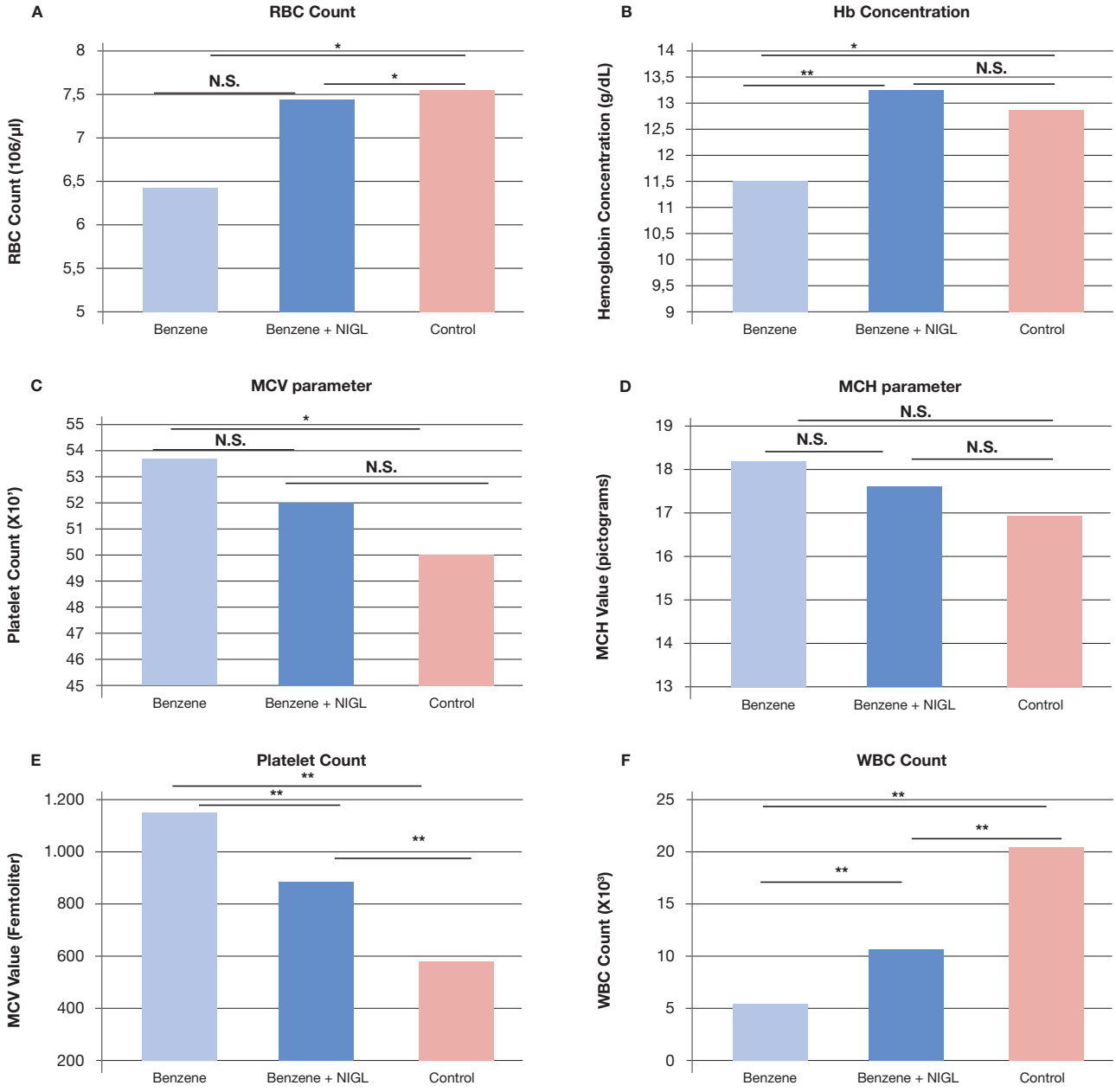

Table II: Significance of changes in Hematological Parameters.

Parameters	Mean value + Standard Deviation			Benzene VS. Control		NIGL+ Benzene VS. Control		NIGL+Benzene VS. Benzene	
	Control	Benzene	Benzene + NIGL	*t- test*	*p* value	*t- test*	*p* value	*t- test*	*p* value
RBCs	7.58 + 0.82	6.40 + 0.58	7.45 + 0.43	3.364	0.004**	0.36	0.72	3.69	0.003*
Hb	12.77 + 1.48	11.50 + 0.27	13.13 + 0.50	2.386	0.031*	0.559	0.58	7.823	0.000**
MCV	49.96 + 3.08	53.62 + 2.83	52.01 + 0.83	2.535	0.023*	1.571	0.14	1.337	0.20
MCH	16.88 + 1.15	18.17 + 1.43	17.65 + 0.39	2.046	0.059	1.534	0.14	0.866	0.40
WBCs	20.06 + 3.33	5.50 + 1.10	10.51 + 0.97	11.76	0.000**	6.748	0.000**	8.84	0.000**
PLT	575.89 + 162.89	1148.88 + 26.50	888 + 19.21	9.8	0.000**	4.614	0.000**	20.34	0.000**

*p-value is significant at the 0.05 level and **.p-value is significant at the 0.01 level.
NIGL denote *N. Sativa*

Discussion

Benzene and benzene-derived agents, widely used industrial chemicals and components of valuable products in modern life, have been reported to induce oxidative DNA damage linked with hematopoietic system disorders malignancies[5,20,28,29]. *N. Sativa* is an antioxidant rich plant and several studies prove its protective effects against oxidative damage mediated by free radicals from chemical agents in animal models[30,31]. However, there is uncertainty about the impact of *N. Sativa* against benzene-induced oxidative DNA damage and toxicity to hematological parameters. The present study is concerned with assessing the potential protective effect of *N. sativa* against benzene-induced DNA damage and hepatotoxicity in rats.

The in vivo models for benzene induced oxidative DNA damage has been established previously by several studies and the increase in the concentration of 8-OhdG is frequently reported as a reliable biomarker for assessing oxidative damage to DNA as well as the risk of cancer development and, therefore, quantifying plasma 8-OhdG is useful for detecting the oxidative stress response induced by benzene in an *in vivo* model[29,32-36]. The results from the analysis of the difference in plasma 8-OhdG levels between study groups revealed consistent finding to what have been previously reported, as demonstrated by the significant increase in plasma 8-OhdG levels in rats exposed to benzene compared to control group, indicating for induction of oxidative stress by benzene in rats[32-37]. Interestingly although the plasma 8-OhdG level was increased in rats co-administered *N. Sativa* along with benzene. However, its level was significantly lower than observed in benzene-exposed rats, demonstrating that *N. Sativa* exerted an antioxidant effect against the oxidative stress response induced by benzene in rats. Many studies supported the beneficial antioxidant effects of *N. Sativa* or its derived compounds in animal models[30,31,38]. These observations suggest the potential benefit of using *N. Sativa* as an antioxidant strategy to manage unfavorable effects caused by exposure to benzene and its derivatives.

To investigate the potential protection role of *N. Sativa* against the toxic effect of benzene toward hematopoietic system, this study evaluated the difference in hematological parameters among rats groups. The analysis indicated abnormal hematological parameters upon administration of benzene as depicted by the significant decrease in RBC count, hemoglobin concentration and increase in platelet count compared to that observed in the control group. The finding agrees with results from several studies that reported benzene-induced hematotoxicity in animal models[32,39,40]. The co-administration of *N. Sativa* extract and benzene had resulted in attenuation of the toxic effect of benzene against hematological cells. In particular, RBC count, Hb concentration, WBC count in rats that co-administered *N. Sativa* along with benzene were significantly higher than that observed in rats treated with benzene only, suggesting that *N. Sativa* could work as an antidote for benzene induced hematotoxicity.

Conclusions

The current study provided evidence that the DNA damage and the hematotoxic effect of benzene can be counteracted by *N. Sativa*. Further experiments are required to study the mechanisms by which *N. Sativa* provides antioxidant stress against benzene-induced oxidative stress.

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