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EFFICIENT SUPPRESSION OF ETHYLENE GLYCOL-INDUCED TOXICITY WITH *NIGELLA SATIVA* OIL IN RATS

Abdullah G. Al-Kushi

Department of Anatomy, College of Medicine,
Umm Alqura University, Makkah, SAUDI ARABIA.
dr.alkushi@gmail.com

ABSTRACT

Ethylene glycol is commonly marketed as "permanent antifreeze". However, its overdose causes a life-threatening toxicity, particularly on brain and bone marrow. Unfortunately, the current treatment of ethylene glycol (EG) toxicity is still non-specific and ineffective, and efforts are being made to seek new alternative therapy. Here we designed a study to evaluate the possible protective role of Nigella sativa oil with potent antioxidant/anti-inflammatory properties, on a rat model of EG-induced toxicity. Forty Wistar rats were randomly and equally divided into 4 groups: Group 1 (control group) didn't receive any medication, while rats of groups 2, 3, and 4 orally received Nigella sativa oil (40 mg/kg), EG (0.1 ml /kg), and EG plus Nigella sativa oil, respectively, for 7 consecutive days. During the study, the animal's survivability was recorded daily. At day 8, all the survived rats were sacrificed and their blood samples, spleens and brains were collected and examined. EG in its overdoses successfully induced severe toxicity in rats with 30% mortality rate. The remarkable metabolic & bone marrow toxic effect of EG toxicity was evidenced by a significant elevation in monocytes count and percentage. Similarly, the histopathological findings confirmed the complete blood count data and indicated the presence of severe injuries in the bone marrow, spleen and brain of rats with EG toxicity. In addition, bone marrow depression, evidenced by decrease in the count of WBCs, RBCs and blood platelets, were significantly observed in rats with EG toxicity. Interestingly, concomitant administration of Nigella sativa oil completely prevented the acute toxic effect of EG overdose-toxicity. Nigella sativa oil supplementation also significantly protected the bone marrow, blood, and brain tissues from the toxic effects of EG. All hematological and histological deteriorations that were associated to EG -overdose-toxicity were significantly reduced with Nigella sativa oil concomitant therapy. Our study provides evidence that Nigella sativa oil supplementation protects bone marrow and brain tissues against EG intoxication and could be used as an effective therapy for this purpose.

Keywords: Nigella sativa oil, ethylene glycol, toxicity, blood, brain, rats

INTRODUCTION

Despite the substantial advances in reduction of human intoxications, the incidence of chemicals and drugs-induced serious organ injury still has a worldwide significance. In this regard, glycolic acid is the major metabolite of ethylene glycol (EG) responsible for toxicity. The three main systems affected by ethylene glycol poisoning are the central nervous system, metabolic processes, and the bone marrow [1]. The central nervous system is affected early in the course of poisoning as a result of direct action of ethylene glycol. Similar to ethanol, it causes intoxication, followed by drowsiness or coma [1].

Seizures may also occur due to direct effect of EG [2]. The toxic mechanism of ethylene glycol poisoning is mainly due to the metabolites produced from ethylene glycol. Initially it is metabolized by alcohol dehydrogenase to glycolaldehyde, which in turn oxidized to

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glycolic acid. The increase in metabolites may cause encephalopathy or cerebral edema [3]. The metabolic effects occur in 12 to 36 hours post ingestion, causing primarily metabolic acidosis which is mainly due to accumulated glycolic acid. Additionally, as a side effect of the first two steps of metabolism, an increase in lactic acid concentration in blood occurs contributing to lactic acidosis. The formation of acid metabolites also causes inhibition of other metabolic pathways, such as oxidative phosphorylation [1].

The spleen toxicity of ethylene glycol occurs in 24 to 72 hours post ingestion, and is caused by a direct cytotoxic effect of glycolic acid. The glycolic acid is then metabolized to glyoxylic acid and finally to oxalic acid. Oxalic acid binds with calcium to form calcium oxalate crystals which may deposit and cause damage to many areas of the body including the brain, spleen, heart, kidneys, and lungs [1]. The rate-limiting step in this cascade is the conversion of glycolic to glyoxylic acid [4]. Accumulation of glycolic acid in the body is mainly responsible for toxicity [5].

Ethylene glycol is not only toxic to humans [6], but also toxic to domestic pets such as cats and dogs. The amount more than 0.1 mL per kg body weight (mL/kg) of pure substance is generally considered as toxic dose. Roughly 16 mL of 50% ethylene glycol for an 80 kg adult and 4 mL for a 20 kg child are toxic enough which require medical intervention. Poison control centers often use more than a lick or taste in a child or more than a mouthful in an adult as a dose requiring hospital assessment [7]. The orally lethal dose in humans has been reported as approximately 1.4 mL/kg of pure ethylene glycol [1], which is approximately 224 mL of 50% ethylene glycol for an 80 kg adult and 56 mL for a 20 kg child. Although survival with medical treatment has occurred with doses much higher than this, death has occurred with 30 mL of the concentrate in an adult [8–10].

Unfortunately, the current medical treatments for ethylene glycol toxicities are often ineffective and non-specific, and therefore efforts are being made to seek new effective medications. Developing pharmacologically effective agents from natural products has become a new trend by virtue of their little toxicity or few side effects. In this regard, a special emphasis has been given on the benefits of *"Herbal Medicine*" as new therapeutic tools and antidotes for various chemicals and drugs-induced toxicities. Among the promising medicinal plants, *Nigella Sativa* or "the black seed", is an amazing herb. In Arabic, it is termed as 'Habbat el Baraka', and has been translated by the prophet "Mohammed" as seeds of blessing having healing powers [11]. *Nigella sativa* has been used in the treatment of a variety of illnesses, including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension, gastrointestinal problems, and eczema. A number of compounds have been isolated from *Nigella sativa;* among them thymoquione (TQ) is considered as the main bioactive component of *Nigella sativa oil.*

Several recent studies have shown that TQ has powerful antioxidant, anti inflammatory, antimicrobial and anticancer effects [12–19]. One of the major mechanisms of TQ is its free radical scavenging properties and inhibition of lipid peroxidation [20]. Recently, an interesting study has been conducted in University of Mississippi Medical Center, USA, which attributed the tissue protective property of TQ mainly to prevention in ROS production and preservation of intracellular antioxidant elements like glutathione [21]. Based on these collective facts, this study was designed and its primary aim was to investigate the possible potential protective role of *Nigella sativa oil* in preventing the brain, blood and spleen cytotoxic events of EG over-dosage and its promising importance.

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MATERIALS AND METHODS

Chemicals

Nigella sativa oil was purchased from Sigma Chemical (St Louis, MO, USA). Ethylene Glycol was purchased from Pharmaceutical markets and suspended in normal physiological saline.

Animals, Treatments and Experimental Design

Forty Wistar rats (weighing 250-300 g) were obtained from the experimental animal house of Anatomy department of Umm Al-Qura University and kept under the same laboratory conditions of temperature (range from 21.9^{o}C to 22.4^{o}C) and lighting (12:12 h light: dark cycle). The rats were given free access to standard laboratory chow food and tap water. They were housed in stainless-steel metabolic cages and allowed to acclimatize for 1 week before experimentation. The rats were randomly divided into four groups (10 rats in each group). Group 1 didn't receive any medication and served as normal control while the rats in group 2 were orally administered (via an intra-gastric tube) with *Nigella sativa* oil (40 mg/kg/day) for 7 consecutive days. Rats in group 3 were intraperitoneal injected by 0.1 ml *Nigella sativa* oil for 2 consecutive days and then orally administered EG via intra-gastric tube with a daily dose of 0.1 ml/kg for 5 consecutive days. Rats in group 4 received EG plus *Nigella sativa* oil daily (by the same administration schedule as in groups 2 and 3) for 7 consecutive days. The doses of EG were selected based on the published reports [53,54] where the remarkable damaging effects of EG on nervous and renal systems were well-documented. The therapeutic doses of *Nigella sativa* oil were also adopted based on earlier studies [11,17]. During the study period, all animal groups were daily examined for their survivability, and the observed mortality cases were recorded. Animals in each group who survived at the end of the study (i.e., at day 5), were finally sacrificed under thiopental general anesthesia. From each sacrificed animal, blood samples were taken from the inferior vena cava; the sample was collected into heparinized microtube (i.e., contained lecithin heparin) for hematological assays. Immediately after blood sampling, the whole spleen and brain were removed quickly and weighed. A part of each isolated organ was prepared for histopathological examination as described below.

All studies were carried out in accordance with the guidelines of the Council of Animal Care, and the protocols were accepted by The Research Ethical Committee of Faculty of Medicine, Umm Al-Qura University in Makkah.

Hematological Assays

The heparinized blood samples were employed for measurement of red blood cell (RBC) count, white blood cell (WBC) count, platelet (PLT) count, hemoglobin (Hb) concentration and hematocrit percentage (Hct%) using an automated hematology analyzing system.

Histopathological Examinations

The animals were sacrificed by an overdose of anesthesia (phenobarbital 60 mg intraperitoneally). Following craniotomy and laparotomy, the brain and spleen were dissected, cleaned of connective tissues and excised from each rat. Each isolated specimens were divided into two parts. The first part was immersed in 10% formol saline, embedded in paraffin blocks and sectioned into 5 microns thick tissue sections. Thereafter, the tissue samples were stained with haematoxylene and eosin (H&E), and histopathological examination were done under a light microscope. The other part was immersed into 2.5% glutaraldehyde, and after multiple steps involving chemical fixation, dehydration and drying,

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the samples were examined using scanning electron microscope (Lice Stereoscan 260 England). The photos were taken.

Statistical Analysis

All data are expressed as mean ± standard error (SE). Data were compared among groups using Student's *t*-test. A *p* value less than 0.05 ($P < 0.05$) was considered to represent a statistically significant difference.

RESULTS

In the present study, we investigated whether co-administration of *Nigella sativa* oil could prevent the acute lethal trend of EG. The administration of toxic EG in toxic doses resulted in 30% mortality rate (3/10) in rats over the study period. However, when we concomitantly administered *Nigella sativa* oil with EG, it fully protected the rats from the acute lethal effect of EG, and no mortality rate was recorded. Therefore, *Nigella sativa* oil can effectively block the lethal effect of EG intoxication.

Table 1. Biochemical and Hematological Effects of Ethylene Glycol (EG) and/or (*Nigella sativa* oil).

Parameter	*Control*	*Nigella sativa oil alone*	*EG alone*	*EG + Nigella sativa oil*
Monocytes	0.23 ± 0.15	0.15 ± 0.04	0.59 ± 0.27*	0.37 ± 0.2#
White blood cells count (10^3/uL)	6.19 ± 00.5	6.77 ± 0.7	2.41 ± 0.1*	5.66 ± 0.2#
Red blood cells count (10^3/uL)	7.22 ± 1	8.63 ± 1.1	3.34 ± 0.4*	6.17 ± 1#
Blood Platelets (10^3/uL)	159 ± 25	160 ± 17	77 ± 9*	120 ± 14#
Hemoglobin) concentration (g/dL)	14.1 ± 2.1	14.9 ± 1.7	5.6 ± 1.3*	12.1 ± 2.1#
Hematocrit value (%)	40.7 ± 2.2	41.42 ± 1.6	15.6 ± 1.8*	34.21 ± 1.1#

* There is significant difference ($P < 0.05$) between values of animals received EG alone and those of normal controls.
# There is significant difference ($P < 0.05$) between values of animals received EG alone and those received EG+ *Nigella sativa* oil.

We observed interesting findings related to hematology. As illustrated in Table 1, in comparison with normal controls, there was a remarkable increase in the number of monocytes and decrease in the number of white blood cells (WBCs), red blood cells (RBCs), and blood platelets (PLT) in the blood of EG-alone received rats. They were also associated with significant decrease in total hemoglobin (HGB) and hematocrit (HCT) values (Tab. 1). However, treatment of these EG-received rats with *Nigella sativa* oil significantly reversed these decrease in WBCs, RBCs, and PLT counts, and corrected the values of HGB and HCT approximately to their normal levels. More interestingly, administration of *Nigella sativa* oil to normal rats produced an increase in blood cellular counts, but it was statistically insignificant.The histopathology of spleen and brain are represented by Figs. 1-6 and Figs. 7-10, respectively.

DISCUSSION

We have designed the study to investigate the effect of *Nigella sativa* oil supplementation on the development of EG over dose intoxication and its associated life-threatening sequels.

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Interestingly, our data showed that concomitant administration of *Nigella sativa* oil successfully prevented the acute lethal effect of EG toxicity and protected rat's spleen, bone marrow and brain from the destructive effects of EG overdose intoxication. The specific protective effect of *Nigella sativa* oil was demonstrated recently [21]. The study reported that reactive oxygen species (ROS) play a major role in the progression of disease. Substances that can attenuate the production of ROS, such as *Nigella sativa* oil, can potentially slow or stop the progression of disease. In acute inflammation, there is an increase in polymorphonuclear cells which lead to increases in local production of H_2O_2, and ultimately to large amounts of ROS and cellular damage. Decreasing ROS will lead to a better solution of acute inflammation [21].

From hematological results, we found remarkable decrease in the number of WBCs, RBCs, and blood platelets in the blood of rats with ethylene glycol overdoses. They were also associated with significant decreases in total hemoglobin and hematocrit which in turn associated with increase in monocytes count. However, treatment of these rats with *Nigella sativa* oil significantly prevented these hematological toxic effects of ethylene glycol, which is in agreement with earlier study [22].

In harmony with our findings, a recent Japanese study has also recorded the same fact in mice, and the authors found that overdose of ethylene glycol resulted in producing some of the toxic agents that have powerful destructing effects on bone marrow DNA. Interestingly, this damaging effect disappeared in *Nigella sativa* oil treated rats. The toxic effect of EG on spleen was reduced spleen cell number [23], histopathological defect and congestion of spleen [24] and spleen lymphatic necrosis [25,26] as in our results. The deposition of crystal occurs in different organs, such as brain and kidney [27] and is possible to occur in the spleen, as seen in the present study. In the rats which received *Nigella sativa* oil, no histopathological change is in agreement with the study by Karawaya *et al.* [28] due to protective effect of *Nigella sativa* oil by preservation of the lymphatic content within the splenic white pulp [28].

We known that ethylene glycol has toxic effect on the human nervous system including generalized seizures and coma [29–35]. Ataxia, slurred speech, confusion [34–40], restlessness, irritation, and disorientation [41–43], and semiconsciousness and unresponsiveness [29,44–47]. During this study, we observed a phenomenon of generalized convulsions in rats which received ethylene glycol, and eventually they died and we did not see other phenomenon such as ataxia and coma. Similar findings are reported by Lyon *et al.* [48], who found a histopathological change in the rat brain with minimum or no neurological symptoms. In histopathological change, the brain necrosis in rat that received ethylene glycol in the present study is in agreement with previous study that showed a brain necrosis in cases of ethylene glycol poisoning [39].

However, a phenomenon of generalized convulsions did not occur in other animal groups including those that received ethylene glycol and simultaneously treated with *Nigella sativa* oil. Therefore, this observation actually reflected the neuro-protective effects of *Nigella sativa* oil against EG-induced neuronal injury in rats. This clinical sign was further confirmed by the histopathological findings. Kanter [49,50] has reported a non histopathological changes of neurodegeneration in hippocampus after chronic toluene exposure in rats by NS and neuroprotective effect on the peripheral nerve. Similar findings have also been reported recently by Kanter *et al*. [51], who found ethylene glycol toxicity-induced hepatoencephally and brain damage and the protective effect of *Nigella sativa* oil against brain damage. In another study by Al-Majed *et al.* [52], neuro protective effect of *Nigella sativa* oil was also

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found in neuronal injury caused by other sources, such as Forebarin ischemia due to chronic toluene exposure.

CONCLUSIONS

The present study indicates that concurrent administration or supplementation of *Nigella sativa* oil can successfully prevent the acute lethal trend of ethylene glycol overdose intoxication as well as its associated serious damaging effects on spleen, bone marrow and brain tissues.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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APPENDIX

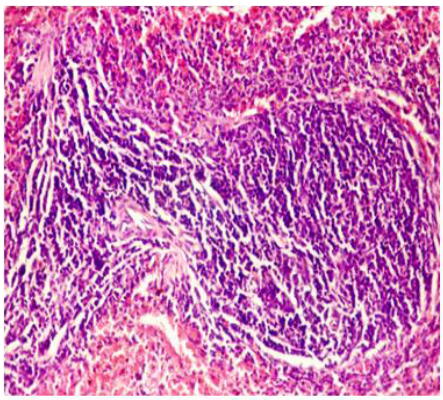

Figure 1

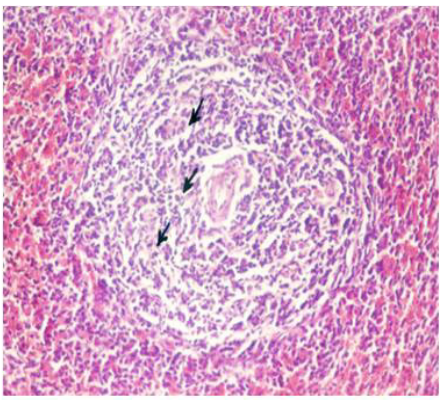

Figure 2

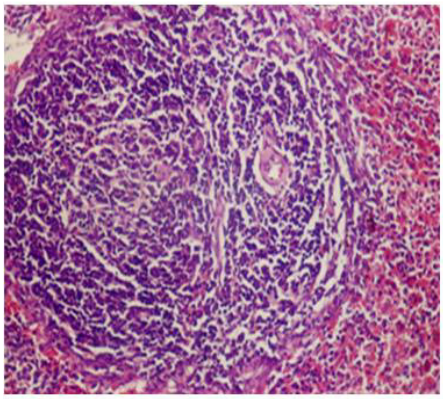

Figure 3

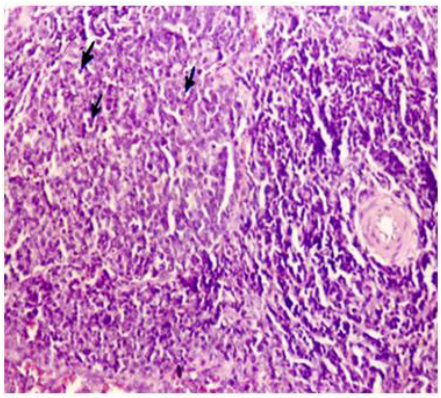

Figure 4

FIGURE LEGENDS

Figure **1** Spleen of control, −ve rats, showing normal lymphoid follicles (H and E × 200).

Figure **2** Spleen of control, +ve rat showing lymphocytic necrosis and depletion (H and E ×200).

Figure **3** Examined spleen section of normal rat treated with nigella sativa oil showing no histopathological changes (H & E × 200).

Figure **4** Examined spleen section of toxic rat treated with nigella sativa oil showing slight lymphocytic necrosis (H and E × 200).

Figure **5** Examined spleen section by scanning electron microscope of normal rat showing no histopathological changes.

Figure **6** Examined spleen section by scanning electron microscope of toxic rat showing crystals deposition on the surface of spleen.

Figure **7** Examined brain section of normal rat showing no histopathological changes (H and E × 200).

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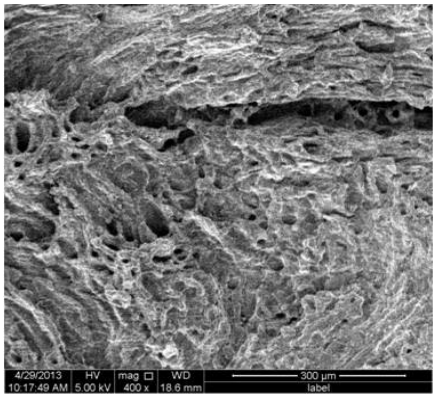


Figure 5

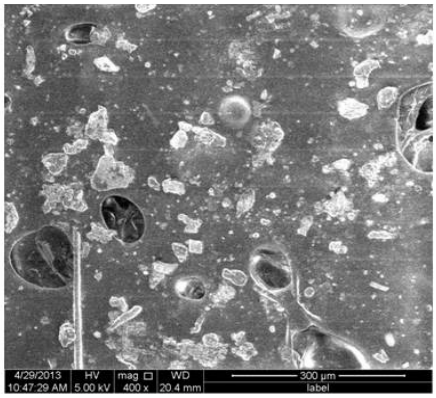
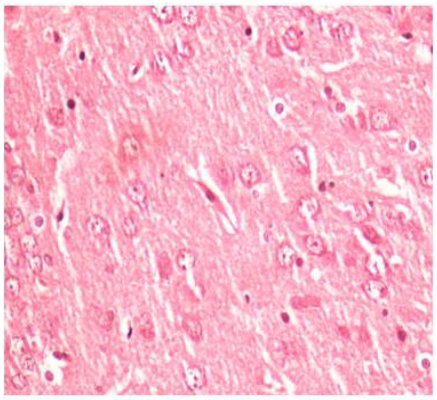


Figure 6



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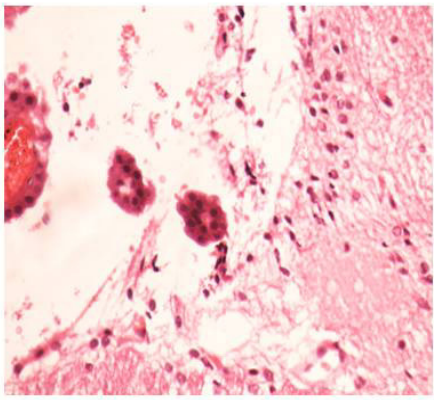

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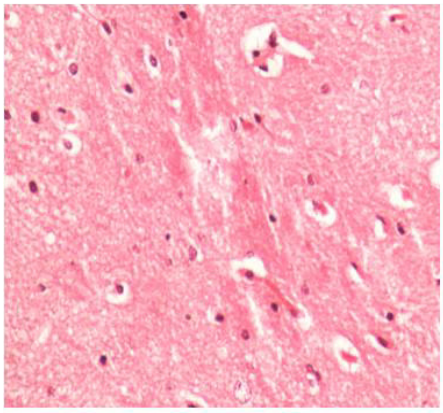

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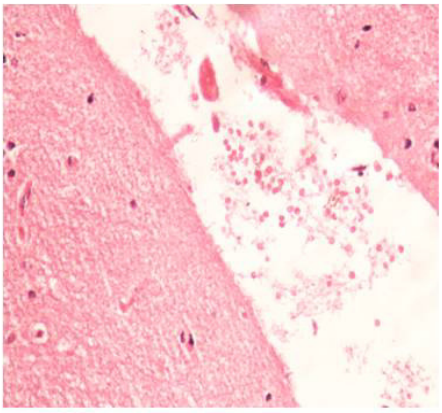

Figure 10

Figure **8** Examined brain section of toxic rat showing slight lymphocytic necrosis (H and E × 200).

Figure **9** Examined brain section of normal rat treated with nigella sativa oil showing no histopathological changes (H and E × 200).

Figure **10** Examined brain section of toxic rat treated with nigella sativa oil showing neither lymphocytic infiltration nor necrosis (H and E × 200).