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The protective effect of wild mustard (*Sinapis arvensis* L.) pollen seeds against the toxicity of a solvent (EGME) in Wistar rats

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Abstract Environmental pollution is the unfavorable alteration of our environment, wholly or mainly as a by-product of human actions, by the direct or indirect effects of changes in energy patterns, radiation levels, the chemical and physical constitution, and the abundance of the organisms. Thus, the environment's pollution is one of the most severe problems humanity and other forms of life face today on our planet, and this population makes severe disruption. The xenobiotic substances responsible for this pollution are numerous and diverse due to multiple human activities that can be the source. However, attention mainly focuses on fertilizers, pesticides, heavy metals, and certain petrochemicals of significant consumption, such as solvents. The purpose of this study is to highlight the reprotoxic and hepatotoxic effects of Ethylene Glycol Monoethyl Ether (EGME) on male Wistar rats. Male Wistar rats were exposed to Ethylene Glycol Monomethyl Ether alone (EGME) (500 mg/kg), combined with the aqueous extract of the pollen grains of *Sinapis arvensis* (P-EGME) (300 mg/kg), in addition to the control (T) and positive control (P) groups for a period of 4 weeks by gavage, to estimate the protective role of this plant against the intoxication of EGME. The results show that EGME can induce reprotoxic effects revealed by a reduction in testes and epididymis mass accompanied by decreased male fertility indicators (sperm concentration). The results also show that treatment with EGME caused a reduction in the red blood cell number, hemoglobin and hematocrit rate, white blood cells in the treated groups compared to the control groups. The present study revealed that treatment with Ethylene Glycol Monoethyl Ether (EGME) under the same experimental conditions could affect several biological markers, especially male fertility. Besides, the protective activity of the wild mustard (*Sinapis arvensis*) pollen in the face of cellular oxidative damage induced by EGME. So, it is suggested that this pollen could be used as a cell protector.

Keywords environmental pollution, ethylene glycol monoethyl ether, hematotoxicity, pollen, reprotoxicity, wild mustard

1. Introduction

Glycol ethers (EGs) are oxygenated solvents whose use has grown widely over the past thirty years. More than thirty glycol ethers are synthesized today by the chemical industry (INRS 2004). EGs have advantageous physicochemical properties, such as low vapor pressure and solubility in mixtures of ethanol and water, making them an excellent solvent for multiple industrial, household, and pharmaceutical applications (Boatman and Knaak 2001). They are well absorbed after dermal, inhalation or oral exposure and rapidly distributed throughout the body, where they exert a toxic influence on reproduction, development, immunological and hematological systems (Barbee et al 1984; Hardin et al 1984; Hardin et al 1984; Lamb et al 1984; Williams et al 1995; Ku et al 1995; Johanson 2000; Udden 2000; Lockley et al 2002; Starek et al 2008; Pomyerny 2014).

An endogenous defense system leads our body made up of amino acids, enzymes, antioxidant substances, among others, capable of eliminating chemical compounds (compounds of solvents). Besides, the organism's endogenous defenses are reinforced by natural antioxidants to prevent lesions' appearance due to oxidation. In this regard, the researchers wondered about the usefulness of various antioxidant molecules in supplementation in the diet, such as some minerals and trace elements (selenium, zinc, manganese, among others), vitamins E and C, and polyphenols (Pastre 2005).

Pollen is a natural product collected by bees from selected flower species, mixed with nectar and bee secretions (Le Blanc et al 2009; Nakajima et al 2009). It has been used as a health food supplement, even used as a medicine for many years (Linskens and Jorde 1997) due to its excellent nutritive properties, including sugars, proteins, fats, vitamins, carbohydrates, and phenolic compounds (Campos et al 2003). Phenolic compounds are one of the most important ingredients linked to the antioxidant activity of pollen. Usually, it contains vanillic acid, protocatechuic acid, gallic acid, p-coumaric acid, hesperidin, rutin, kaempferol,

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apigenin, luteolin, quercetin, and isorhamnetin (Bonvehí et al 2001; Chu et al 2007).

This composition tends to be species-specific and linked to the therapeutic properties (antibiotic, antineoplastic, anti-diarrheal, and antioxidant) of pollen (Campos et al 1997; Almaraz-Abarca et al 2004; Mărghitaş et al 2009).

Our work aims to determine the effects of EGME administered by gavage on male reproduction (concentration and mobility) and some hematological parameters in Wistar rats. On the other hand, we have tried to determine the phytochemicals of aqueous extract of pollen grains of wild mustard (*Sinapis arvensis* L.) and their protective effect against the toxicity induced by this solvent.

2. Materials and Methods

2.1. Biological material

In this study, we used 32 male rats from the Pasteur Institute in Algiers. These rats were subjected to an adaptation period of about one month to the biology department animal facility (room temperature and natural photoperiod). The rats are kept in plastic cages. The cages were cleaned, and the litter was changed every three days until the end of the experiment. The animals had *ad libitum* access to water with food containing all the necessary elements (maize, soybeans, minerals, and vitamins) from Oued Fragha-Guelma. Each group was assigned the same amount.

2.2. Chemical material

A solvent from the family of oxygenated solvents was used: ethylene glycol monoethyl (%), obtained from Biochem chemopharma. EGME was dissolved in water and then administered orally at a dose of 500 mg/kg BW.

2.3. Vegetal material

The plant, which was the object of our study, is known under the name Field mustard (*Sinapis arvensis* L.), from which we used the pollen grains. Our samples' collection was carried out during April, at the El Chafia region level (Wilaya of ELTarf).

2.3.1. Preparation of the extract

The aqueous pollen grain extract of Wild Mustard (*Sinapis arvensis* L.) was made in the form of a suspension. Daily, the pollen grains were ground with a commercial grinder, then mixed with distilled water and administered orally at 300 mg/kg BW.

2.4. Experimental protocol

The thirty-two male rats were divided to have an average weight per equal group, into six experimental groups of eight rats each (n = 8) and received the following treatments:
-The control group (T): received tap water as drinking water.
-The positive control group (P): received orally 1 ml of an aqueous suspension of wild mustard pollen (*Sinapis arvensis* L.) grain at a rate of 300 mg/kg BW.
-The group (EGME): rats treated orally with 1 ml of ethylene glycol monoethyl at a rate of 500 mg/kg BW.
-The group (P-EGME): rats treated with a mixture of the aqueous suspension of pollen grains of wild mustard (*Sinapis arvensis* L.) (300 mg/kg BW) with EGME (500 mg/kg BW).

The rats are sacrificed by cervical decapitation without anesthesia to avoid keeping them in a state of stress.

2.5. Sample collection

2.5.1. Blood sample

The blood was immediately collected in tubes containing an anticoagulant (EDTA) to measure the hematological parameters.

2.5.2. Dissection

After the dissection, the testes and epididymis were removed and stripped of their fatty tissue, then weighed using a precision balance (Kern PRS 320-3).

2.6. Study of hematological parameters

The measurement of the blood count formula was carried out using an automatic hematology machine (Brand: ABACUS 4). The EDTA tube containing the blood is placed in the machine, and the SNSF measurement begins. After 2 minutes, the results appear on the screen, and then we print them out. The hematological parameters measured were red blood cell (GR), white blood cell (GB), hemoglobin (Hb), hematocrit (HT).

2.7. Study of the biology of spermatozoa

After dissection, the sperm is taken from a small opening in the epididymis's tail to study: the concentration and mobility of the sperm. 1 µl of semen is diluted in 50 µl of physiological Na Cl 0.9% solution.

2.7.1. The concentration and mobility of sperm

The latter is measured using the sperm class analyzer (SCA) by putting a drop of the sperm into the Goldcyto blade (made up of several counting chamber formats). This preparation was examined under a microscope-integrated into the microcomputer at a final magnification x4, programmed to calculate the sperm concentration and motility.

2.8. Statistical study

Data were expressed as mean±SEM. Statistical analysis and graphs were performed with Prism7. The one-way analysis of variance (ANOVA) test was used to compare more than two groups. The criterion of statistical significance was set at $P < 0.05$.

3. Results

3.1. Absolute testis weight

Rats intoxicated with EGME show a very significant decrease (*P* < 0.001) in the absolute testicular weight compared to the control and positive control groups. In contrast, oral administration of the aqueous extract of pollen grains (P) to EGME poisoned animals significant (P < 0.05) increased weight and testes compared to animals from the EGME (Figure 1).

3.2. Sperm concentration and motility

The sperm concentration was highly significantly (*P* < 0.01) decreased in the rats in the (EGME) group compared to controls and positive controls. This concentration increased in a highly significant way (*P* < 0.01) in the animals of the group (P-EGME) compared to those of the group (EGME) (Figure 2). No significant change (*P* < 0.05) was recorded in sperm motility in our study's different groups (Figure 3).

3.3. Hematological study

3.3.1. Number of red blood cells

Treatment of rats with EGME induced a significant decrease (*P* < 0.05) in the number of red blood cells compared to control and positive control rats, as well as a non-significant reduction (*P* > 0.05) in rats treated with the combination aqueous extract of pollen grains and EGME comparing to rats treated with EGME alone (Figure 4).

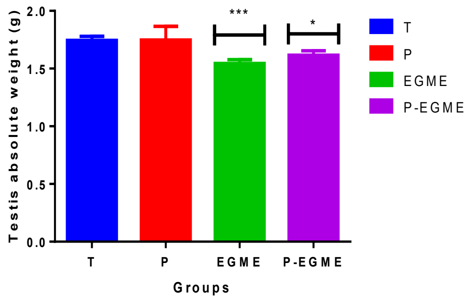

Figure 1 Changes in the absolute weight of testis (g) in the groups of rats: T, P, EGME, P-EGME (n = 8). * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

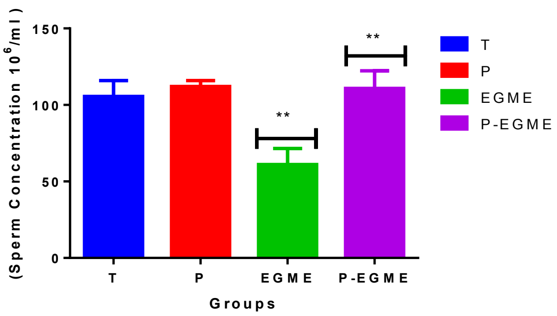

Figure 2 Variation in the concentration of spermatozoa (10^6/ml) in the groups of rats: T, P, EGME, P-EGME (n = 8). * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

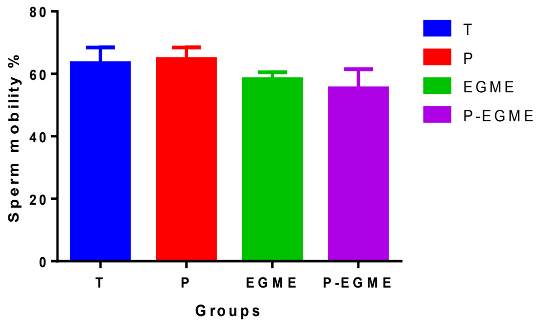

Figure 3 Variation in sperm motility (%) in the groups of rats: T, P, EGME, and P-EGME (n = 8). * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

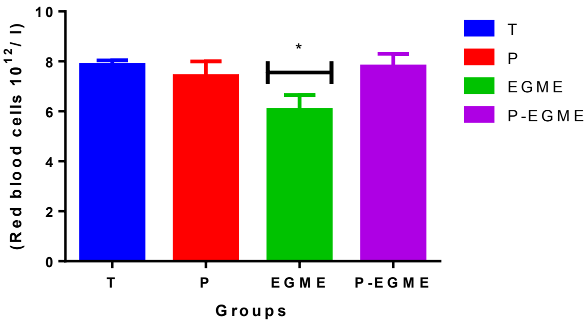

Figure 4 Variation in the number of red blood cells (10^{12}/l) in the groups of rats: T, P, EGME, and P-EGME (n = 8). * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

3.3.2. Hemoglobin level

We find a significant decrease (*P* < 0.05) in hemoglobin level in the animals of the (EGME) group compared to the intake in the (T) (P) groups. On the other hand, the combination of aqueous extract of pollen grains and EGME increased in a highly significant way (*P* < 0.01) this hemoglobin level compared to those of the group (EGME) (Figure 5).

3.3.3. Hematocrit level

Our results show a significant decrease (*P* < 0.05) in the hematocrit level of the group's animals (EGME) compared to controls and positive controls. This drop in the hematocrit level increased significantly (*P* < 0.05) in animals in the (P-EGME) group compared to those in the (EGME) group (Figure 6).

3.3.4. Number of white blood cells

From the results obtained, there was a significant increase (*P* < 0.05) in the number of white blood cells in the animals treated with EGME compared to controls and positive controls. This considerable increase improved significantly (*P* < 0.05) in animals poisoned with EGME and treated with the aqueous extract of the pollen grains compared to animals treated with EGME alone (Figure 7).

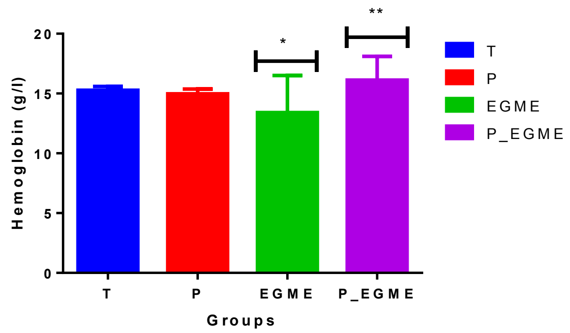

Figure 5 Change in hemoglobin level (g/l) in the groups of rats: T, P, EGME, and P-EGME (n = 8). * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

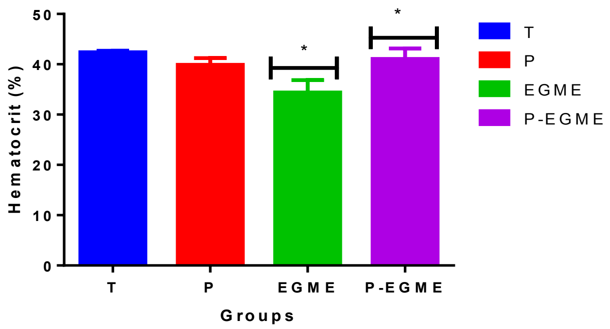

Figure 6 Variation in the hematocrit level (%) in the groups of rats: T, P, EGME, and P-EGME (n = 8). * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

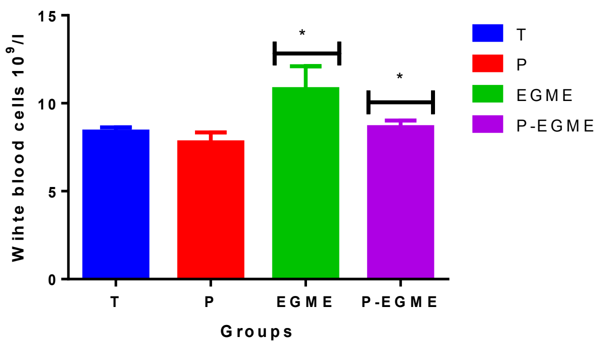

Figure 7 Variation in the number of white blood cells (109 / l) in the groups of rats: T, P, EGME, and P-EGME (n = 8). * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

4. Discussion

Since ancient times humankind has used various plants in their habitats to treat and cure all kinds of diseases. These plants represent a very vast reservoir rich in potential compounds attributed to "secondary metabolites", which have the advantage of having a great diversity of chemical structures and possessing an enormous range of biological activities (Laouar 2018).

In recent years, attention has been focused on one of the medicinal plants' biological activities, the antioxidant activity. This attention is due to the role on plays in the prevention of chronic diseases by combating the oxidative stress induced by several factors such as pollution, smoking, aging, and psychological stress but especially intoxication by xenobiotics (Boelsterli 2007; Lodovici and Bigagli 2011; Verschuere et al 2012; Aschbacher et al 2013; Meddour et al 2013; Siriwardena 2014).

Glycol ethers represent a diverse series of dexenobiotics with properties that make them widely suitable for various solvent applications. Their hydrophilic and lipophilic properties have wide industry applications (Johanson 2000; Bagchi and Waxman 2008). On the other hand, these compounds or their metabolites can induce many pathological cases, particularly hematopoietic, reproductive and developmental toxicities (Bagchi and Waxman 2008).

In this study, we investigated the toxic effect of EGME on reproductive and hematological parameters. Besides, we investigated the protective effect of aqueous extract of wild mustard (*Sinapis arvensis*) against EGME toxicity.

Reproduction is an important biological function that ensures continuity and biodiversity. It is a fundamental process that allows living organisms to conserve their offspring and evolve by passing on genes (Fujii et al 2003). Epidemiological data have shown that the quality of human sperm has declined over the past 60 years, while the incidence of abnormalities of the male genital tract and infertility has increased (Auger et al 1995; Miguel et al 2006; Phillips and Tanphaichitr 2008).

The decline in sperm quality observed in men developed over a short period, suggesting that it could result from environmental factors (Phillips & Tanphaichitr, 2008). Among the factors that affect male fertility and normal sperm parameters, chemical compounds play an essential role (Tas et al 1996). Many chemicals used for other conditions can negatively affect sperm motility and viability (Hargreaves et al 1998).

Our study results show a considerable decrease in the absolute weights of the testes and epididymis of rats treated with EGME compared to the control and positive control groups. A similar study showed the same observations in rats treated with 200 mg/kg BW EGME over 40 days (Adeyemo-Salami and Farombi 2018). Exposure to glycol ethers can cause deleterious effects on the structure and function of the sexual organs (Holloway et al 1990).

Microscopic examination of a histologic study of the testes and epididymis revealed pathologic lesions associated with treatment at 400 mg/kg BW EGME. The testes treated with EGME showed severe congestion and bleeding in the interstitium seminiferous tubules, severe erosion of the germinal epithelium of almost all seminiferous tubules, severe necrosis of germ cells with a significantly reduced Sertoli cell population (Adedara and Farombi 2010).

EGME has also been shown to cause gonadotoxicity by inducing oxidative stress in testes and epididymis, as demonstrated by the effect on membrane components by lipid peroxidation, which is an indication of oxidative degradation of lipids by free radicals (Adeyemo-Salami and Farombi 2018).

The evaluation of the effects of EGME on the quality and quantity of sperm showed a significant decrease in some biological parameters of the sperm (concentration and rate) in rats treated with EGME alone compared to controls.

The observed decrease in sperm concentration may indicate that EGME affects the early stages of spermatogenesis, which is well supported by the observed reduction in daily sperm production (Wang et al 2004; Welsch 2005).

The first wave of spermatogenesis in rats is triggered when the gonocytes first differentiate into spermatogonia three days after birth; these first become spermatocytes at around day 9, form haploid spermatids by day 18 form the first spermatozoa at day 43 (de Rooji 1998). Each phase of spermatogenesis has different susceptibilities to various toxins. Often, exposure to a specific toxicant targets a spermatogenesis phase, depending on the toxicant mode of action (Adedara and Farombi 2010).

Ethylene glycol mono-ethyl ether has been reported to negatively affect early and late pachytenes and dividing spermatocytes (Watanable et al 2000). It has also been reported to reduce testicular testosterone, which regulates both Sertoli and germ cells during spermatogenesis (Reader et al 1991). As another significant testicular toxicity mechanism, the effect of oxidative stress on testicular toxicity must be taken into account (Yamamoto et al 2007).

An earlier study marked reactive oxygen species (ROS) involvement in infertility due to impaired sperm function (Aitken et al 1987; Agarwal et al 2014). Adeyemo-Salami and Farombi (2018) reported that oral administration of EGME at 400 mg/kg BW in adult male rats for 15 days causes a disturbance in enzymatic and non-enzymatic antioxidants, with an increase in the rate of lipid peroxidation; this could alter sperm morphology by affecting components of the sperm membrane, such as phosphocholine, phosphatidylcholine, and lipids (Yamamoto et al 2007).

EGME's effect on sperm motility may be due to their action at the middle part and flagellum. These two areas are the parts that ensure the movement and speed of the sperm (fertility indicators). Foster et al (1983) showed that the first visible lesion in pachytene spermatocytes is in the mitochondria and that damage to spermatids with elongated nuclei results in reduced sperm movement; this may explain the decrease in lactate production in the Sertoli cell (Mebus et al 1989). The latter is considered the key enzyme to provide the energy necessary for sperm speed and mobility by converting pyruvate to lactate attached by NADH oxidation to NAD and catalysis by lactate dehydrogenase (LDH) (Miki et al 2004; Miki 2007).

The hematologic system can be considered a conductor for substances that enter the body and as an organ that can be affected by exposure to potentially harmful agents. Blood samples can serve as biological control of exposure and provide the means to assess a toxicant's effects on the hematopoietic system and other organs. Agents in the environment can interfere with the hematopoietic system in several ways: by inhibiting hemoglobin synthesis, as well as cell production or function, and by accelerating the destruction of red blood cells (Dufresne 2000).

This study shows the effects of EGME on the blood compartment. Based on the results obtained, a decrease in the erythrocyte count, hematocrit, hemoglobin level, and white blood cells was observed in rats exposed only to EGME. Therefore, these observations are consistent with Bendjedou and Khelili (2014), who found that treating male rabbits at 300 ppm EGME for four weeks corroborates our conclusions.

Based on these observations, the authors suggested that the decline in blood elements could be attributable to hemolytic anemia (cell lysis) caused by the toxin's ability to act directly on erythrocytes causing massive extravascular hemolysis or d '' induce oxidative stress (Hashish and Elgaml 2016; Lalruatfela et al 2014; Demur et al 2013).

Although devoid of mitochondria, red blood cells or erythrocytes are susceptible to free radicals (Demur et al 2013; Brun et al 1998). Oxidative stress can alter the erythrocyte membrane by attacking membrane lipids, thereby making cell membranes more fragile and causing red blood cell lysis (Hashish and Elgaml 2016; Lalruatfela et al 2014).

EGME may also impact the immune system, resulting in decreased thymus weight, thymic atrophy, and a reduced number of spleen cells (Exon et al 1991).

In this study, therapeutic intervention in EGME poisoned rats with *Sinapis arvensis* pollen grain extract at a 300 mg/kg BW dose effectively attenuated the deleterious effects of EGME on reproduction, restoring the weight of reproductive organs with concomitant improvements in the concentration and mobility of sperm. Besides, the co-administration of aqueous extract of wild mustard pollen grains was found to be effective in protecting hematological parameters against the detrimental effect of EGME, thereby increasing cellular defense processes against -vis the cytotoxic effects of free radicals.

The observed therapeutic potency of wild mustard (*Sinapis arvensis*) pollen grains could be due to several contributing factors, including phenolic compounds, flavonoids, and carotenoids (Abdeldjalil 2014). These compounds have been shown to reduce lipid peroxidation and LDL oxidation levels by removing free radicals from hydroxyl and superoxide anions (Tiwari 1999; Jovanovic and Simic 2000). Besides, stimulate transcription of SOD and catalase genes (Toyokuni et al 2003; Ranaivo et al 2004), thus increasing antioxidant activities and reducing oxidative damage induced by free radicals in human cells.

5. Conclusions

Administration of EGME 500 mg/kg BW to rats for 30 days caused a metabolic disturbance as evidenced by the onset of impairment of reproductive and hematological parameters. This disturbance is associated with structural alteration of the testes, epididymis, and bone marrow. Besides, it is responsible for the appearance of significant changes induced by oxidative stress that disrupt the enzymatic detoxification systems and the body's defensive capacities.

Through our study, our results also show that the administration of the aqueous extract of the grains of wild mustard pollen (*Sinapis arvensis*) (300 mg/kg BW) to animals treated with EGME leads to the improvement of most of the parameters studied.

The results of this study seem to suggest that wild mustard (*Sinapis arvensis*) provided a protective effect against reprotoxicity and haemato-toxicity induced by EGME, and this thanks to its composition in polyphenols and flavonoids responsible for the antioxidant potential of this plant.

Conflict of Interest

The authors declare that there are no conflict of interest with this work.

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