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The toxic effects of the ethylene glycol monomethyl ether (EGME) in male rabbit

Mouna Bendjeddou and Kamel Khelili

Laboratory of Animal Ecophysiology, Department of Biology, Faculty of Sciences, University Badji Mokthar, El Hadjar, Annaba, Algeria

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ABSTRACT

In order to evaluate the effects of the toxicity of EGME, an experimental study has been carried in laboratory of animal echophysiology on 30 male rabbits (Oryctolagus Cuniculus). These latter was treated with EGME introduced by cutaneous way (intradermal injection) with two increasing doses 200 and 300 ppm for 4 successive weeks. The chemical treatment with the EGME causes a variation in the hematological and biochemical parameters: the significant disturbance in the cellular parameters of blood which include the numeration of blood cells (white blood celles, red blood cells, hemoglobin, hematocrit , the mean corpuscular volume and mean corpuscular hemoglobin) in individuals treated by the EGME compared to the witnesses ,an increase in the weight of liver was observed especially in rabbits treated by important doses. In addition, the serum concentration in glucose was significantly dcecreased compared with the witness to all treated rabbits. A significant increase in the rate of cholesterol, triglycerides and total protein was detected in rabbits treated by report to the witnesses. Finally, the obtained results highlight a reduction of the rate of glutathione GHS in studying organs such as the liver, testes and epididymis. According to our results we can conclude that the exposure to the EGME causes important biological variations.

Key words: Ethylene Glycol Monomethyl Ether (EGME), rabbit, *Oryctolagus Cuniculus*, toxicity, glutathione

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INTRODUCTION

The pollution is an unfavorable modification of the natural environment , it can affect man directly in his health or more distant his environment [1] .

Xenobiotic substances responsible for this pollution are many and varied because of multiple human activity that can be the source, but the attention is particularly concerned fertilizers, pesticides , heavy metals, petrochemicals and some consumer products such as solvents [2] .

These have known a large human use in the industry without considering their adverse effects on health [3], for that purpose several researches study the effect of solvents on the physiology of the human beings as the Ethylene glycol mono methyl ether that is one of these products .

Glycol ethers are oxygenated solvents whose use have greatly expanded over the past thirty years. More than thirty glycol ethers are synthesized by the chemical industry today . They are divided into two series: ethylene derivatives (E series) and derivatives of propylene glycol (P series) . Until 1990, derived from ethylene glycol were the main glycol ethers marketed , probably because ethylene oxide required for their synthesis is an important under product of the petroleum Industry [4] . Their low acute toxicity compared to most organic solvents favored their presence in many preparations for industrial and domestic use such as paints, inks, varnishes, stains, cleaning products, liquid soaps, cosmetics or certain pharmaceutical formulations [5]. For this, man may be exposed and in direct contact with

these products in his occupational environment , so he stays in a situation of "threatened" for decades , at the same time the industries that manufacture, process and use ether glycol can transmit it into the air and release into surface water or subterranean [6] .

The penetration of glycol ethers in the human body is mainly through making during a direct contact, through the skin , their low volatility limit the contamination by respiratory way except in situations where the products are warmed or used in the form of aerosols.

Because of their amphiphilic character, glycol ethers cross easily membranes and are divided into the aqueous and lipid compartments. Significantly absorbed regardless of the pathway (oral, dermal , lung), they are distributed in most biological tissues , including fetal tissues. The enzymatic systems transform these glycol ethers into hydrosoluble compounds more easily eliminated or into reactive metabolites responsible of toxic manifestations [7 , 8] .

Most experimental studies have determined the toxicity and the impact of these products on health [9] , their metabolites appear to be more toxic than their initial products [10] .

In this axis moves our work which bases it self on an experimental study of the effect of a solvent : Ethylene glycol monomethyl ether on some biochemical and hematological parameters in male rabbit of the local race *Oryctolagus Cuniculus*.

MATERIALS AND METHODS

Animals and Treatment

This research has been carried out in the laboratory of animal echophysiology , on thirty domestic male rabbits of the local population *Oryctolagus Cuniculus* (ages of 06 to 09 months, body weight 1700 ±130 g) from the region of Annaba. The rabbits are stirred in the conditions of our pet center, the food was well balanced and varied, it contains all the necessary elements for the natural growth of animals: salad, carrots, hard bread crushed and mixture of corn and wheat, the same quantity is provided in the waterers cages which is changed each day. The rabbits were treated daily by tracks cutaneous (intradermal), by two different doses of EGME (200 and 300 ppm) for 4 weeks. These animals were divided into 3 lots : 2 batches treaties and one other witness. The taking of the blood and the organs has been done after the decapitation of rabbits at the end of treatment. The biochemical assays and hematological are achieved using kits of commerce ready to use (Spinreact kits are used for determinations of glucose and total proteins; however, the Biosystems kits are used for the assay of triglycerides and cholesterol). The tissue glutathione is determined by spectrophotometry according to the method of Weeckbeker and Cory [11] (the reagents come from the firm Sigma).

Hematological and biochemical parameters

The blood collected in EDTA tubes is used for the counting of blood cells (white blood cells, red blood cells) , the rate of hemoglobin, the hematocrit , the the mean corpuscular volume, and the mean corpuscular hemoglobin ,by means of Coulter Counter Rubis. Three other fractions of blood are collected in héparines tubes, then spin-dried, the obtained plasma is aliquot and preserved at a temperature of-20°C for the dosage of proteins, glucose, cholesterol, triglycerides with the methods of [12,13].

Statistical analysis

The results are represented in the form of mean Â± standard deviation (m ± SD), using the Student *t*-test , using Minitab software version (15).

RESULTS

Physiological Study

Liver weight

The variation of the weight of the liver in treating rabbits with 200 and 300 ppm of Ethylene glycol mono methyl ether for 4 weeks reported to the weight in witnesses , showed a significant increase in the liver weight among the two lots treaties. Figure1.

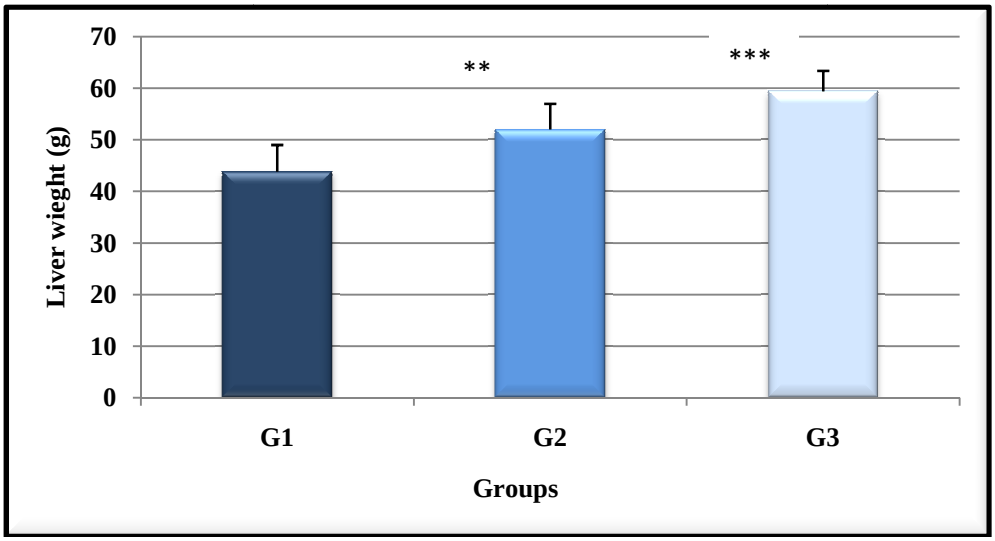

Figure 1 : Variations of the liver weight in rabbits witnesses and treated dailywith 200 and 300 ppm of EGME during 4 weeks
(each value represents the mean Â± DS of 10 rabbits).
*(p< 0.01) **, (p< 0.001) ****

Biochemical Study

The results of the assay of glucose, triglyceride, cholesterol and protein totals are shown in table 1. The results show a significant decrease in concentrations of glucose which is proportional each time at the dose of the EGME administered.

The increase of the concentration of cholesterol ,triglycerides and total protein is highly significant in the two batches treaties.

Table 1 : Determination of biochemical parameters in rabbits exposed to EGME
each value represents the mean Â± DS of 10 rabbits

Parameters	Witness	G1 (200 ppm)	G2 (300 ppm)
Glucose (g/L)	1.35 ±0.04	0.74 ±0.08 ***	0.66 ±0.04 ***
Triglyceride (g/L)	1.51 ± 0.05	1.59 ± 0.04 *	1.7 ± 0.05 ***
Cholesterol (g/L)	0.15 ± 0.03 ***	0.26 ± 0.04 ***	0.58 ± 0.05
Total protein (g/L)	10.7 ± 0.51	13.78 ± 0. 40 ***	15.57 ± 0.30 ***

*(p<0.05) *, (p< 0.01) **, (p< 0.001) ****

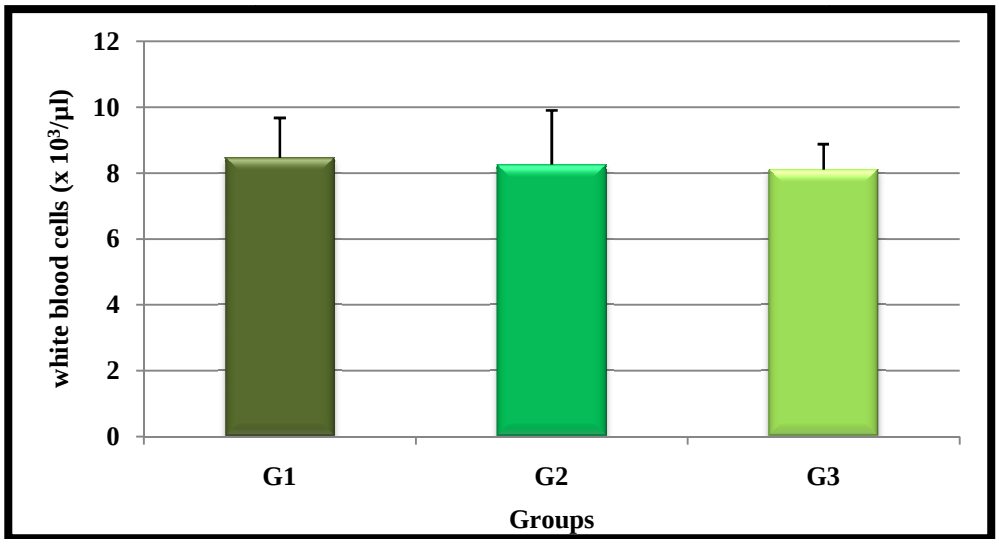

Figure 2: Variations in the number of white blood cells (n GB) in the control group and the treated groups with EGME

Hematological Study

The results obtained are shown in figurs (2, 3 , 4, 5, 6, 7) and reveal that there is a significant change of the number of red blood cells , hemoglobin, hematocrit , the mean corpuscular volume, and the mean corpuscular hemoglobin among all lots processed by report to the batch indicator.

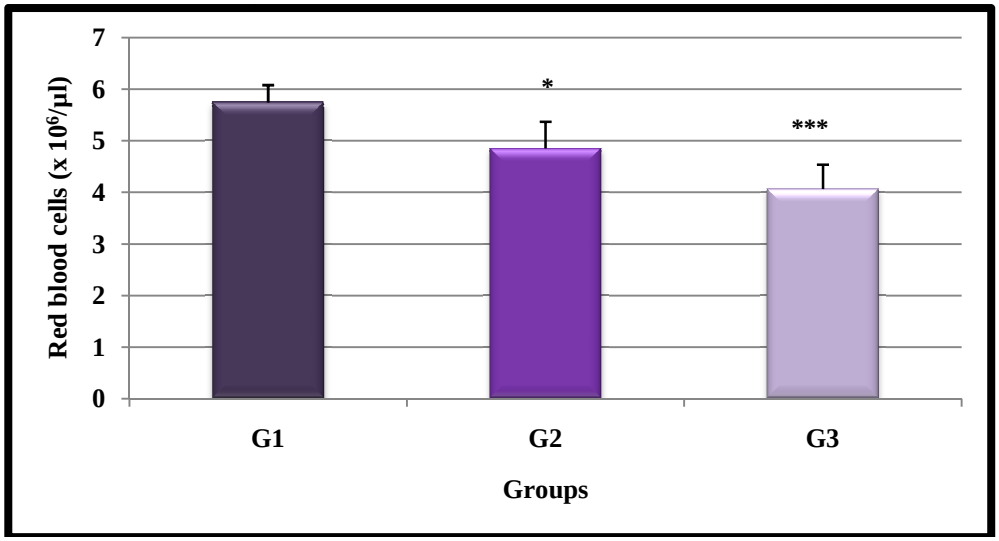

Figure 3: Variation in the number of red blood cells (n GR) in the control group and the treated groups with EGME
*(p<0.05) *, (p< 0.001) ****

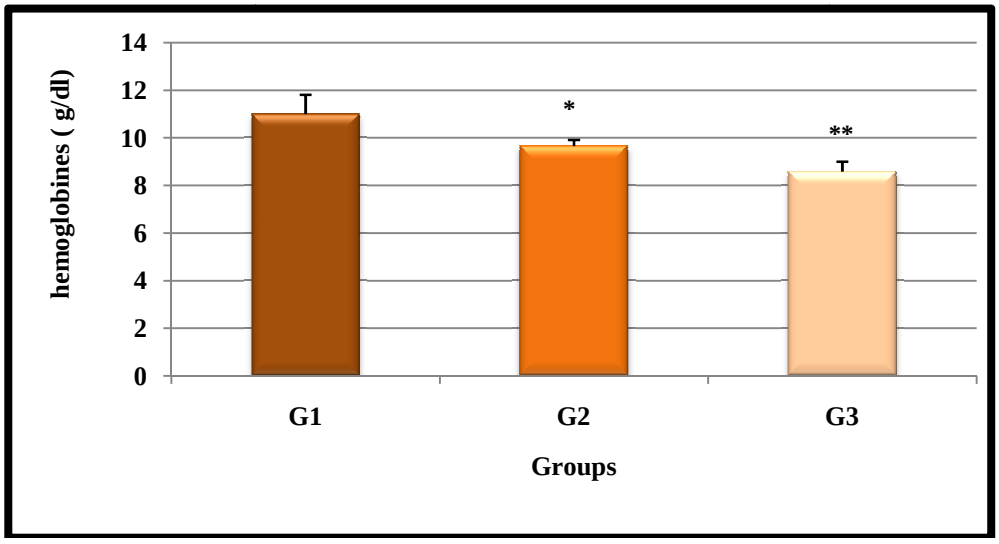

Figure 4: Variation in the number of hemoglobin (Hb) in the control group and the treated groups with EGME
*(p<0.05) *, (p< 0.01) ***

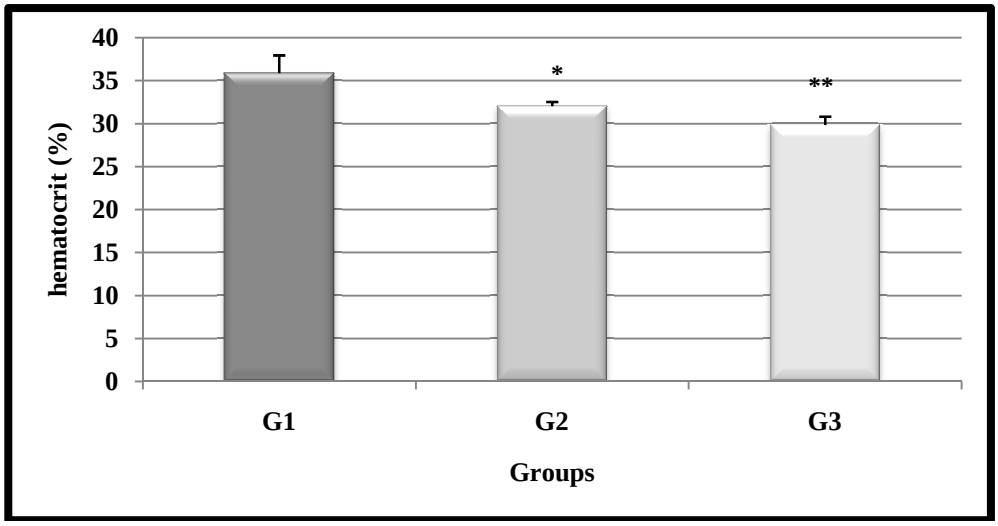

Figure 5: Variation in the number of the percentage of hematocrit (Ht) in the control group and the treated groups with EGME
*(p<0.05) *, (p< 0.01) ***

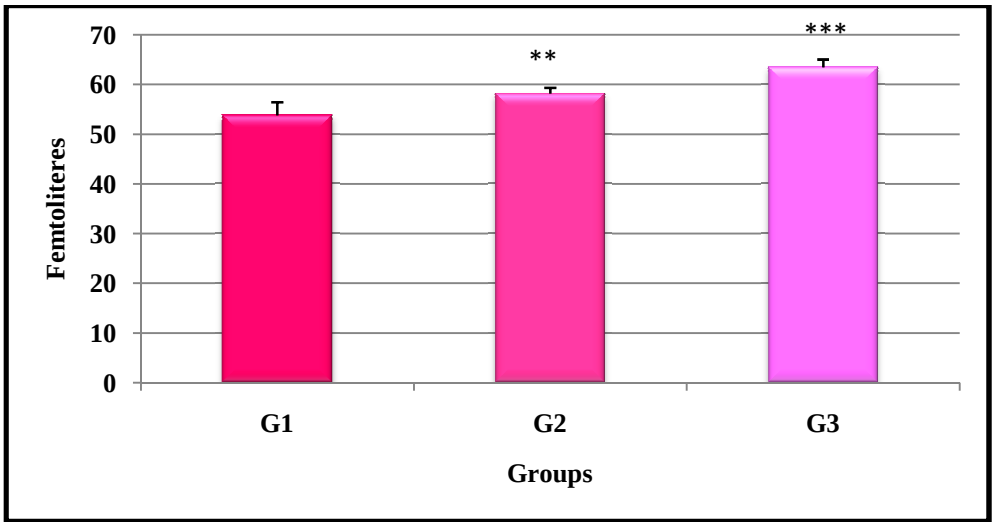

Figure 6 : Change in average mean corpuscular volume (MCV) in the control group and the treated groups with EGME
*(p< 0.01) **, (p< 0.001) ****

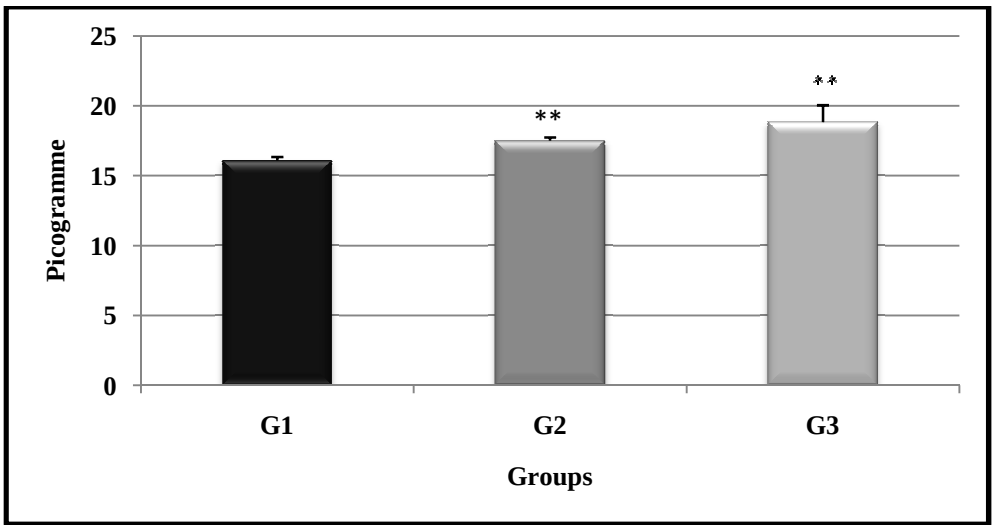

Figure 7 : Change in mean corpuscular hemoglobin (MCH) in the control group and the treated groups to EGME
*(p< 0.01) **

Toxicological Study

The evaluation of the toxicity of EGME is expressed by the dosage of the intracellular glutathione at the level of the various target organs; the results are represented in table 2. The glutathione decreased significantly at the level of all the organs studied: the liver, testes and epididymis.

This decrease is proportional to the doses of the pollutant administered and especially at the level of target organs such as the liver and testes.

Table 3 : Rate of glutathione (Nm/mg protide) in rabbits witnesses and treated daily with 200 and 300 ppm of EGME during 4 weeks

each value represents the mean Â± DS of 10 rabbits

Bodies	Witnesses	G1 200 PPM	G2 300 PPM
Liver	57.11 ± 1.94	49.4 ± 1.39***	44.1 ± 1.85***
Testicle	28.53 ± 2.67	23.98 ± 1.22*	22.94±2.16 **
Epididymis	0.53 ±0.04	0.43 0,05*	0.40 0,05*

*(p<0.05) *, (p< 0.01) **, (p< 0.001) ****

DISCUSSION

Most of the chemicals intended to be used in industrial domain areas have toxic effects on biochemical and hematological parameters in humans and animals. Ethylene glycol monomethyl ether as an example of study exerts its toxicity through acidic metabolites and more aldehydes. These are capable of penetrating into the nucleus and altering the structure and the functioning of the genome regulating cell growth and development.

Our results revealed a disruption of metabolic products, we noticed the increase of the rate of cholesterol ,triglycerides and plasma proteins, as well as a decrease in the rate of glucose level in treating individuals. All these disturbances are due to the change of the functions of the body by the metabolites of the EGME [5].

The repeated administration by ethers of glycol sometimes produces hepatic functional and / or histological changes. They are always benign (modulation of enzymatic activities, hepatocytes swelling , increased liver weight) and translate rather an adaptive metabolic answer than a hepatic toxic effect. They are described below, for each compound successively with which they have been reported.

The Hepatic effects reported with EGME were observed for doses higher than those susceptible to induce hematological disorders. Heinonen and Vainio (1981) [14] exposed Wistar rats 6 hours a day, 5 days a week for 2 weeks at 0 , 50, 100 or 400 ppm of EGME , they observed an increase in the concentration of glutathione reduces the activity of hepatocytes and hepatic UDPglucuronyltransférase with a parallel decrease in the activity of the NADPH-cytochrome c reductase . In a series of experiments , Kawamoto *et al* showed , in Wistar rats receiving 0 , 100 or 300 mg / kg / day of EGME , per OS ,during 20 days , an increase of the activity of hepatic gamma-glutamyltranspoptidase , from 100 mg / kg / day [15, 16] ; an increase in the activity of the cytosolic alcohol dehydrogenase was also observed at 300 mg / kg [17, 18] , there was no significant effect on the cytochrome P450 , the cytochrome b5 and the NADPH -cytochrome c reductase [17, 18] .Miller *et al*. (1983 and 1984a). [19, 20] exposed rats and rabbits at 0.30 , 100 or 300 ppm of EGME 6 hours a day, 5 days a week for 13 weeks , they observed a decrease in liver weight of animals exposed to 300 ppm , this anomaly is related to the global weight reduction in rats and rabbits observed from 100 ppm and does not reflect a specific liver toxicity.

Concerning the evaluation of hematologic toxicity , we recorded a significant decrease in hemoglobin, the red blood cells, the hematocrit and a significant increase in the mean corpuscular volume and the mean corpuscular hemoglobin, with a non significant decrease in the rate of white blood cells. This toxicity has been widely studied on animal models : hemolysis [21, 22, 23] , Lymphocytic deletion responsible fo immunosuppression [24, 25] and a toxicity and myeloid progenitors of the bone marrow [26] .

The haemolytic effect of the ethylene glycol derivatives , in particular the EGBE (Ethylene Glycol Butyl Ether) , depends on the dose of the product , the animal species , and within the same species , the age of animals [27] . These are not the substances mothers who are responsible for hemolysis , but the alkoxyacétiques acids is (AAA) . Many studies have revealed that ethers glycol of the E series have the property of destroying red blood cells. An Exposure of rats to EGnPE in the dose 200 ppm orally during 11 days causes hemolysis [28] . The same result obtained with a dose of 30ppm of the EGIPE by inhalation for 28 days [29] and 100 ppm for 3 weeks [30] in rats . Regarding the toxicity of the bone marrow , the studies carried out on laboratory animals [26] have shown that ethylene glycol derivatives , especially those with short chain alkyl (EGME) are responsible for hypocellularity (a decreased blood lines), a decrease of granulocyte progenitors and erythrocyte in particular. These effects are responsible for leukopenia with neutropenia , and anemia.

The mechanism of bone marrow toxicity of the ethers glycol was little studied , some works showed that ethers of glycol can inhibit the DNA synthesis and / or cause abnormal mitotic spindle in the marrow precursors . The immunosuppressive effects are mainly related to lymphocyte depletion and less important functional impairment of lymphocytes [24].

Generally speaking, human red blood cells seem to be less sensitive . For other ethers of glycol of the same series , an achievement of the cells of the bone marrow accompanied with a decrease in the number of white blood cells was observed.

In addition, some of these ethers of glycol cause a lymphocyte depletion [8], a fall in hemoglobin [21] with a reduction of the blood counts for the EGBE [31].

The toxicity of the ethers of glycol is clearly less well documented in humans and in animals , the likely exception of cases of medullary hypoplasia affecting primarily the neutrophil lineage. She still has unknowns importance. Hemolysis appears to be very rare, except in cases of mass poisoning. Regarding toxicity on the bone marrow, ethers of glycol seem to be responsible for neutropenia and rarely aplastic . However, their potential liability in the induction of hematologic malignancies does not appear documented.

Facing the oxidative damage caused by free oxygen radicals and the toxic effects caused by active metabolites of xenobiotics , the living cell can defend itself through several detoxification systems , the most important is that of glutathione [32] . This importance results in his great ability to detoxify many harmful compounds that can be endogéniques (active metabolites of hormones) or exogéniques (toxic metabolites of xenobiotics) .According to the results obtained , the treatment of rabbits with doses 200 and 300 ppm for 4 weeks caused a decrease in liver , testicular and epididymal content glutathione .the GSH plays a key role in the detoxification of free radicals and heavy metals [33] , And in the case of the EGME , it interacts directly with high affinity for thiol groups (- SH) of GSH , glutathione may also interact with the free radicals generated by this metalloid [34 , 35, 36 , 37] .The Ethylene glycol monomethyl ether inhibits the glutathione synthetase and the glutathione reductase, GSH is so little product. All these factors lead to a strong decrease in the reduced glutathione (GSH) and the oxidized glutathione increased (GSSG) , and consequently a reduction in the activity of GSH - dependent enzyme .

GSH can interact directly with activated oxygen species but it is mainly used as a substrate for glutathione peroxidase which ensures the elimination of lipid peroxides [38, 39]. it forms the first line of defense by acting as a non - enzymatic by direct interaction of the group with sulfur or ROS may be involved in the reaction of the enzymatic detoxification of ROS [40].

CONCLUSION

The results of this study demonstrate the toxic effects of ethylene glycol monomethyl ether (EGME) administered to rabbits by cutaneous way. The Toxicity was evaluated by disturbances in many body functions:

- A Hematotoxicity resulted in a significant decrease in hemoglobin, the red blood cells, the hematocrit and a significant increase in the mean corpuscular volume, and the mean corpuscular hemoglobin.
- A disturbance in the rate of the metabolic products (hepatotoxicity).
- A Decrease of the detoxifying potential of the organism.

From these results, it would be important to be interested in the characterization of the toxicity of the product in humans through the implementation of epidemiological studies in professional circles.

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