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## Systemic Effects of Ethanolamine Oleate: An Experimental Study

MOHAMED S. HAFEZ, M.D.; EFFAT EL-SHERIF, Ph.D.  
and AIDA A. KHATTAB, Ph.D.

*The Departments of Surgery, Pathology and Pharmacology,  
Faculty of Medicine, Cairo University.*

### Abstract

An experimental study for the systemic effects of 5% ethanolamine oleate was conducted on 46 rats, divided into 3 main groups. Rats in group A were subdivided into 3 subgroups of 5 animals each. They were injected once intraperitoneally with 2, 4 and 5 ml of the drug respectively. None of the animals showed any sign of acute toxicity. Histological examination was performed 24 hours after the injection, to the liver, spleen, kidneys, heart, lungs, brain, blood and bone marrow and it did not reveal any abnormality. Group B included 28 rats, which were studied for the chronic effect of the drug. They were sub-divided into 4 subgroups, which were injected daily with 0.5 ml of 5% ethanolamine oleate for 2, 4, 6 and 8 weeks respectively. None of the animals died. Histopathological changes were found only in the lungs, affecting 37.5% of the injected animals. There was lymphoid hyperplasia in 20.8%. Interstitial and intraalveolar oedema and haemorrhage, with vascular congestion were detected in 25%. There was no correlation between the duration of the injections and the incidence of the lung lesions. Group C included 3 rats, which were injected once in the lower oesophagus through a laparotomy. They were given 0.25, 0.5 and 0.75 ml of the drug respectively. Only the rat which was injected with 0.75 ml, developed lung oedema, haemorrhage and congestion, when examined 2 weeks after the administration. It can be concluded that 5% ethanolamine oleate is relatively a safe drug. Rats tolerated up to 5 ml single dose without any detectable effect. This dose is equivalent to 180 ml if given to man. However, with frequent repeated doses, there is a potential hazard of injury to the lung capillaries.

### Introduction

ENDOSCOPIC injection sclerotherapy has become a widely accepted treatment modality for the control of bleeding oesophageal varices. Unlike the injection of varicose veins, large volumes of the sclerosing solutions are used. Between 40 to 50 ml of the sclerosant are required for the

perivariceal sclerotherapy per session [1]. Up to 30 ml were injected into the varices in the intravariceal technique [2, 3]. Besides, injection is repeated, usually at 1-3 week intervals, until the varices are eradicated [1,4,5]. Systemic dissemination of the sclerosing solution has been proved, particularly with the intravariceal injection [6-11]. In spite of all these facts, the safety of

the recommended dosages has not been adequately elucidated.

Five per cent ethanolamine oleate is one of the commonly used sclerosing solutions. It is the sclerosant available in Egypt. This work is an experimental study for the systemic effects of this drug. It also aims at finding out the organs most vulnerable to its toxicity.

#### Material and Methods

This experimental study was conducted on 46 adult, male, albino rats weighing about 200 gm. The drug to be examined was 5% ethanolamine oleate. The animals were divided into three main study groups:

##### *Group A: Acute Toxicity Study:*

The aim of this part of the work was to define the LD<sub>50</sub> of the drug. Fifteen rats were subdivided into 3 subgroups. Four animals of each subgroup were injected once intraperitoneally with 5% ethanolamine oleate and they were observed for signs of toxicity [12]. Subgroup A<sub>1</sub> were injected with 2 ml, subgroup A<sub>2</sub> were injected with 4 ml, while in subgroup A<sub>3</sub> 5 ml were used. The fifth rat in each subgroup was left as a control and was injected with saline in equal volume to the drug. All animals were sacrificed after 24 hours.

##### *Group B: Chronic Toxicity Study:*

This experiment included 4 subgroups of 7 rats each. Six animals of each subgroup were injected daily intraperitoneally with 5% ethanolamine oleate in a dose equivalent to the maximum dose used in man in the variceal sclerotherapy. The latter is 30 ml [2,3]. By the use of Paget and Barnes [13] converting table, the dose to be given to a rat was about 0.5 ml. The seventh rat of each subgroup was left as a control and was injected daily with 0.5 ml sa-

line. The daily injection was carried out on in the subgroups B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and B<sub>4</sub> for 2, 4, 6 and 8 weeks respectively. The animals were sacrificed by the end of the injection period.

##### *Group C: Oesophageal Injection Study:*

In order to mimic the procedure of variceal sclerotherapy in man, the study was extended to include the systemic effects of 5% ethanolamine oleate when injected in the lower oesophagus of rats. Three animals were anaesthetised by intraperitoneal injection of 10 mg of thiopental. Through a small laparotomy the drug was injected once. The first rat was injected with 0.25 ml, the second with 0.5 ml and the third with 0.75 ml. The three animals were sacrificed after 2 weeks.

All the rats, included in the study, were killed by bleeding. The liver, spleen, kidneys, heart, lungs and brain were fixed in 10% formalin for histopathological examination. Also a blood smear and a bone marrow sample were examined in each rat.

#### Results

##### *Group A: Acute Toxicity Study:*

None of the 15 rats of this group showed any sign of toxicity. There were no histological abnormalities in all the examined tissues.

##### *Group B: Chronic Toxicity Study:*

None of the 28 rats of this group died during the study. Microscopically, only the lungs of some of the animals injected with ethanolamine oleate were affected. In subgroup B<sub>1</sub>, the lungs of 2 rats (33.3%) showed lymphoid hyperplasia (Fig. 1). In subgroup B<sub>2</sub> lymphoid hyperplasia was found in one rat (16.7%). In another rat there were interstitial and intraalveolar haemorrhage and oedema, with vascular

congestion (Fig. 2). The lung changes in subgroup B<sub>3</sub> involved 3 animals (50%). There were oedema, haemorrhage and congestion in the three and together with lymphoid hyperplasia in two of them. Oedema, haemorrhage and congestion were also observed in 2 rats of subgroup B<sub>4</sub> (Table 1).

*Group C: Oesophageal Injection Study:*

The rat which was injected with 0.75ml of the ethanolamine oleate developed interstitial and intraalveolar oedema and haemorrhage and vascular congestion of the lungs.

### Discussion

Rapid systemic dissemination of the sclerosing solution during intravascular sclerotherapy has been proved by fluoroscopic visualization [6,7], as well as by scintillation camera [8-11]. Such spread occurred regardless of the injected volume [8,11]. Entry of the drug into the pulmonary circulation was documented by the radionuclide studies [8-11]. Connors et al. [9] estimated that approximately 20% of the solution migrated to the lungs. Richards et al. [10] found high radioactivity in the lungs in all of 19 positive scans.

Table (1): The Incidence of Lung Changes in the Chronic Toxicity Group and Subgroups.

Group	Lymphoid hyperplasia	Hemorrhage, oedema and congestion	Total number of affected rats
B <sub>1</sub>	2 (33.3 %)		2 (33.3 %)
B <sub>2</sub>	1 (16.7 %)	1 (16.7 %)	2 (33.3 %)
B <sub>3</sub>	2 (33.3 %)	3 (50.0 %)	3 (50.0 %)
B <sub>4</sub>		2 (33.3 %)	2 (33.3 %)
Total B	5 (20.8 %)	6 (25.0 %)	9 (37.5 %)

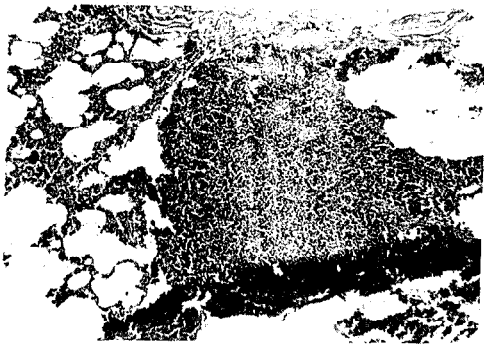


Fig.(1): Lung lymphoid hyperplasia in a rat of group B.

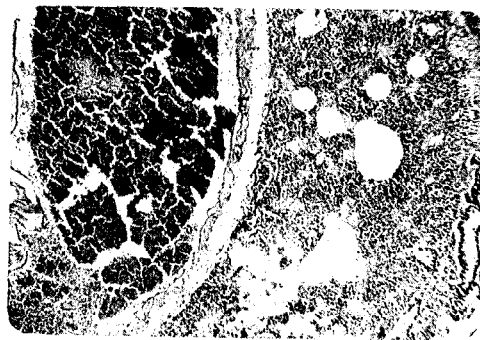


Fig.(2): Lung of a rat of group B showing interstitial and intraalveolar hemorrhage with vascular congestion.

While local complications of variceal sclerotherapy has been well studied, systemic adverse effects were only presented as sporadic case reports. After the use of 5% ethanolamine oleate, anaphylactic reaction [14], haemolysis [15], gross haematuria [16] and acute renal failure [15,17] have been recorded. Coronary artery spasm with transient ECG changes was also noted [18]. This could be due to increased sympathetic activity under that stressful circumstances. Nevertheless, rise in serum creatine phosphokinase after the injection of ethanolamine oleate was observed before [19]. There were also patients who developed fatal cardio-respiratory failure, but this was after the use of polidocanol [20,21] and sodium morrhuate [22]. Kanai et al. [23] presented a case of fatal progressive pulmonary fibrosis subsequent to the injection of 30 ml of ethanolamine oleate 5%.

Transient mild impairment of the haemostatic process occurred shortly after sclerotherapy [24-26]. This was attributed, however, to consumption of the clotting factors [25,26]. As a matter of fact, there are many distant complications, which are outside the interest of this work, since they were due to extension of the local effect of the sclerosant or complications of the procedure.

An experimental study [27] was conducted on rats using oleic acid, which is the main constituent of ethanolamine oleate. There was approximately 7 folds increase in pulmonary vascular permeability after intravenous injection of 0.25 ml of 20% oleic acid. Interstitial and intraalveolar oedema and hemorrhage and vascular congestion started after few hours and were most pronounced after 24 hours. These lung changes disappeared within 12 days. Multiple injections of oleic acid did

not result in chronic lung damage. The same lung changes were detected in rabbits after a single intravenous administration of 100 mg/kg oleic acid [28] and 125 mg/kg [29].

Hitherto, to the best of our knowledge, there is no experimental studies dealing with the systemic complications of ethanolamine oleate. The present trial corroborated the previous studies on oleic acid in the occurrence and in the pathology of lung lesions. Our results, however, differed in five points:

- 1- Intraperitoneal administration of 5% ethanolamine oleate in single, different doses failed to find out the LD<sub>50</sub>. Up to 5ml were injected without even detectable effect. This dose is equivalent to 180 ml if given to man [13]. The LD<sub>50</sub> of pure oleic acid, when given intravenously, was 0.06 ml [27], which is equal to 1.2 ml of 5% dilution.

- 2- The single intraperitoneal doses of 5% ethanolamine oleate did not give rise to overt lung pathology, when examined 24 hours after the administration. The lung injury after intravenous oleic acid, on the contrary, had a maximum peak after 24 hours [27-29].

- 3- Unlike the results of oleic acid studies, the lung lesions were inconstant among our animals. Only 15.5% of the 39 rats injected with ethanolamine oleate developed detectable lung changes.

- 4- Once of the three rats, which were injected once into the oesophagus and which were sacrificed 2 weeks later, showed lung oedema and hemorrhage Dickey et al. [27] previously reported that the lung changes had subsided within 12 days only.

5- There was no record in the oleic acid studies on the lung lymphoid hyperplasia seen in some of our animals.

Interpretation and analysis of these differences will not be valid, since the present study differed in the drug used, concentration, dosages and mode of administration. On the other hand, the similarities in the induced lung pathology suggests that the oleic acid component of ethanolamine oleate is the radical responsible for the injury.

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