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The Effect of Moclobemide, Reversible Inhibitor of Monoamine Oxidase-A, on the Alcoholized Rat Brain

by *Erdal Karaöz*[1], *Mehmet Kanter*[2] *& Zihni Bahçi*[3]
[2]Department of Histology and Embryology, Süleyman Demirel University Faculty of Medicine, Isparta, Turkey. e-mail: ekaraoz@hotmail.com [1]Department of Histology and Embryology, Yüzüncü Yil University Faculty of Veterinary Medicine, 65080 . Kampus, Van, Turkey mehmetkanter65@hotmail.com [3]Department of Pharmacology, Gülhane Military Medical Academy, Ankara, Turkey.

Summary
This experiment was carried out to demonstrate the effect of moclobemide on the brains of rats fed on a diet containing alcohol. Thirty male rats, 200-250 g were used. Rats were fed with a diet (milk) containing ethyl alcohol (10%) in the alcohol-only treated group and were injected subcutaneously with moclobemide (30 mg/kg) in the alcohol+moclobemide treated group daily for 21 days. It is found that the serum ethanol level in the alcohol+moclobemide treated group was significantly higher than in alcohol-only treated group at the end of the experiment. Electron microscopic examination revealed more prominent neurotoxicity in the alcohol+moclobemide treated group than in alcohol-only treated group. We concluded that moclobemide decreases the elimination of ethanol. However, more studies are needed to demonstrate its mechanism.

Introduction
Ethanol is a central nervous system (CNS) depressant. Chronic use of ethanol by humans results in a variety of psychological and physical dysfunctions including a decrease in ability to perform cognitive tasks, loss of ability to form new memories, and cerebral atrophy (*Sheetz et al.,1988*). Chronic alcohol ingestion also results in massive cerebral and cerebellar cortical degeneration in rats (*Paula-Barbosa et al.,1989*).
Moclobemide is a new, reversible and selective MAO-A (Monoamine oxidase-A) inhibitor with fewer side effects and has antidepressant properties (*Berlin et al.,1990*). Moclobemide is safer and more tolerated than other MAO-A inhibitors such as, clorgyline, brotamine, isocarboxaside and harmaline (*Stefanis & Merz,1990*). In humans, moclobemide is rapidly absorbed after a single oral administration and maximum concentration in plasma is reached within an hour. It is strongly bound to plasma proteins. Eighty percent MAO-A inhibition occurs in two hours; the duration of MAO inhibition is usually between eight to ten hours (*Nair et al.,1993*).
In this study, we investigated the possible interaction between moclobemide and alcohol. Change in the serum ethanol level was measured and pathological alterations in neural tissues were examined. Brain has been chosen as a histological model to demonstrate the degree of alcohol toxicity.

Materials and Methods
Thirty male Wistar rats weighing approximately 200-250 g. and 20 days old, were used. The rats were divided into three groups (Control, Alcohol-only treated and Alcohol+Moclobomedie treated). Each group consisted of ten rats placed in two cages. Rats were fed *ad libitum* with milk for a month (Table 1). It is suggested that milk is the ideal liquid diet for administering alcohol *(Parale & Kulkarni, 1986)*. After a month of milk feeding, ethanol (99.9%) at 10% v/v was added to the milk of both the was added at 10% v/v to the alcohol-only and alcohol+moclobemide treated groups for

Table 1. The constituents of milk

Substance	Amount
Milk lipids	40.7 g/l
Dextrin-maltose	50.0 g/l
Colin bitartarate	0.15 g/l
Vit B1	0.14 mg/l
Vit B2	1.5 mg/l
Vit B6	0.7 mg/l
Nicotinic acide	0.9 mg/l
Ca pantotenate	3.0 mg/l
Folic acide	0.05 mg/l
Biotine	0.05 mg/l
Vit B12	7.0 mg/l
Vit A	1025.0IU/l
Vit D	14.0IU/l
Vit E	1.0 mg/l
Vit K	60.0 mg/l
Ca	1250.0 mg/l
P	920.0 mg/l
Na	500.0 mg/l
K	1500.0 mg/l
M	120.0 mg/l
Mn	10.0 mg/l
Cu	5.0 mg/l
	0.3 mg/l
Zn	4.0 mg/l
I	0.047 mg/l
Cl	1000.0 mg/l
Se	0.03 mg/l
SO4	1000.0 mg/l
Cr	0.01 mg/l
Inositol	20.0 mg/l

21 days. In addition Moclobemide (Roche) was injected subcutaneously to the animals in the mocolobemide+alcohol treated group for 21 days.

Drug used: A solution (0.3%) of moclobemide (Roche) was prepared in distilled water and injected (subcutaneously) at the dose of 30 mg/kg every day (*Da-Prada & Burkard, 1990; Schoerlin & Da-Prada, 1990*).

Analysis of serum ethanol

Serum ethanol level was analysed, by using fluorescence immunoassay method, in 3 ml. blood taken from the animals the end of the experiment.

Electron microscopic examination

Brain tissues including frontal cortex and medulla from the right hemispheres of the rats were fixed in 2.5% glutaraldehyde and were postfixed in 1% osmium tetroxide in phosphate buffer. Following dehydration, tissues were embedded in Araldite CY 212. Thin sections (500-600 A°) were prepared from araldite blocks using a LKB Nova Ultramicrotome and stained with aqueous uranyl acetate and Reynold's solution and examined and photographed under a Carl Zeiss Em 9S2 electron microscope.

Statistical Analysis

Data were expressed as mean ±SEM. Student's t-test was used to demonstrate a significant difference between the alcohol-only and alcohol+moclobemide treated groups. In all cases a $P<0.001$ was considered to be significant.

Results

Serum-ethanol level

Serum ethanol levels of alcohol-only and alcohol+moclobemide treated groups are presented in Fig. 1. The ethanol level of the alcohol+moclobemide group was significantly higher than that of the alcohol-only treated group ($P<0.001$).

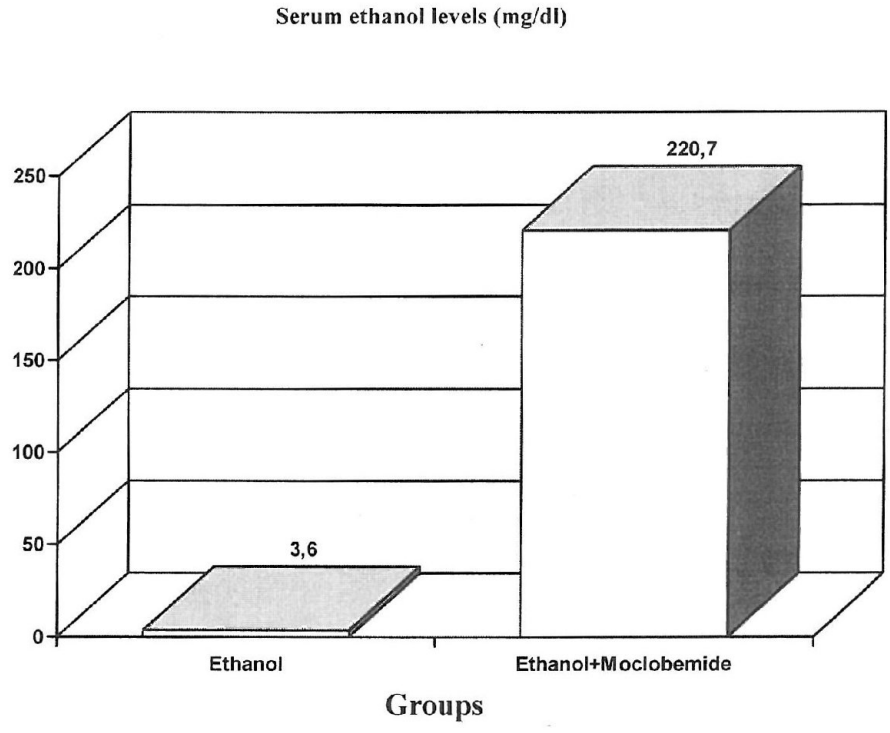

Fig. 1. Histogram of serum ethanol concentrations in alcohol-only and alcohol+moclobemide treated groups.

Histological observation

The histological examination of the brain of animals in control group showed normal structure and no lesion was seen.

On the other hand, when the frontal cortex neurons of the alcohol-only treated group were examined, cellular and nuclear membranes were found to be damaged. The cytoplasmic vacuolar degeneration, dilatation of the endoplasmic reticulum cisternae, increased lysosomal dense bodies and lipid droplets, and destruction of the mitochondrial cristae were observed (Fig. 2,3). Some findings were observed in the glial cells. Most importantly, degenerative myelin sheaths of the medulla

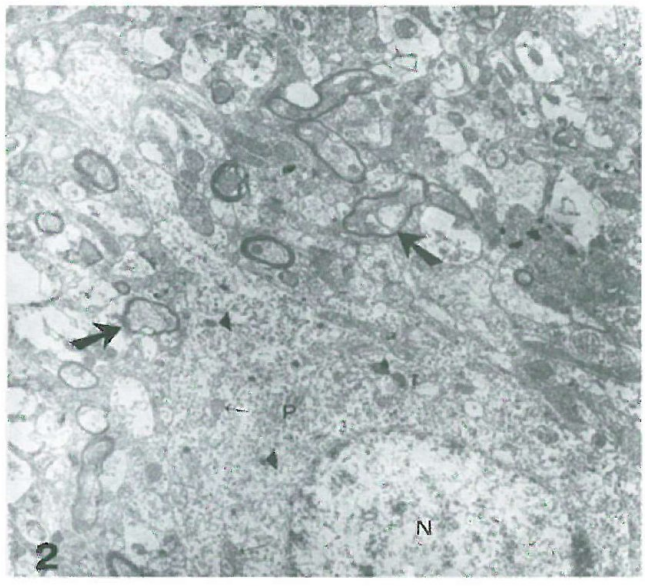

Fig. 2. Electron micrograph of a rat brain 21 days after ethanol liquid diet. Perikarion (P), nucleus (N), mitochondria (thin arrows), lysosomal dens bodies (arrowheads), myelin sheath (thick arrows). Uranyl acetate and Reynold's lead stain (X10000).

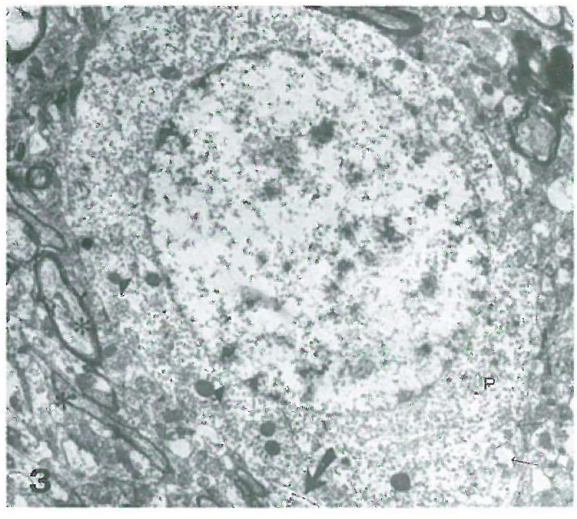

Fig. 3. A transmission electron micrograph of the frontal cortex from alcohol-only treated group. The presence of the dilatation of the endoplasma reticulum (thick arrow), crista disruption in mitochonodria (thin arrows), lysosomal dens bodies (arrowheads) in the cytoplasm of a perikaion (P) are shown. Furthermore, degenerative myelin sheath (asterisks) are present. Uranyl acetate and Reynold's lead stain (X16000).

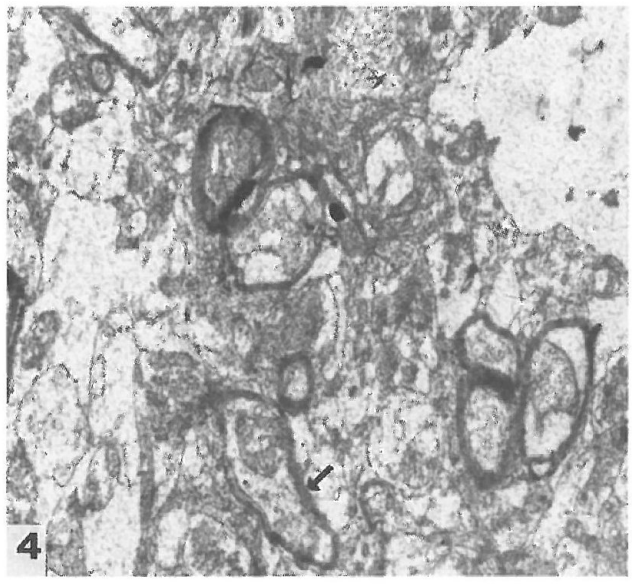

Fig. 4. Transmission electron micrograph of medulla from alcohol-only treated group. Myelin degeneration in the axon (arrows). Uranyl acetate and Reynold's lead stain (X10000).

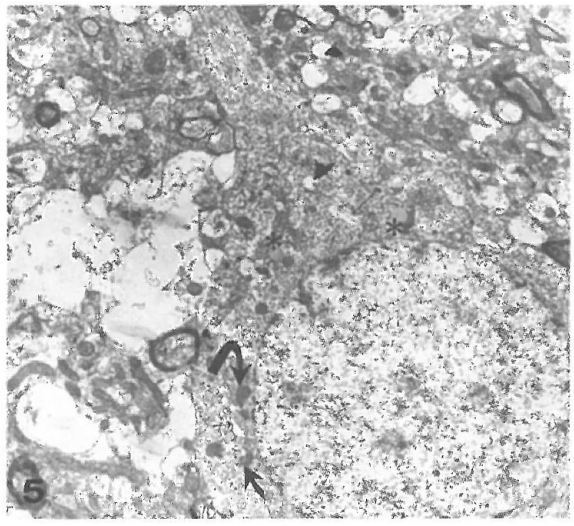

Fig. 5. The cytoplasmic degenerations including dilatation of endoplasma reticulum cisternae (thin arrows), crista disruption in mitochondria (arrowheads), and increased lysosomal dens bodies (thick arrows) and lipid droplets (asterisks) are observed in the alcohol–moclobemide treated group. Uranyl acetate and Reynold's lead stain (X15000).

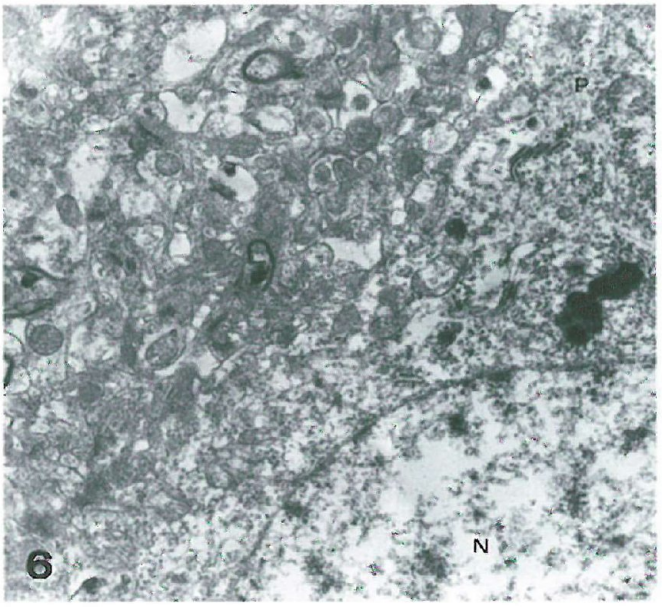

Fig. 6. Cellular and nuclear membrane destruction of a neuron from frontal cortex were observed in the alcohol+moclobemide treated group. Perikarion (P), nucleus (N). Uranyl acetate and Reynold's lead stain (X17500).

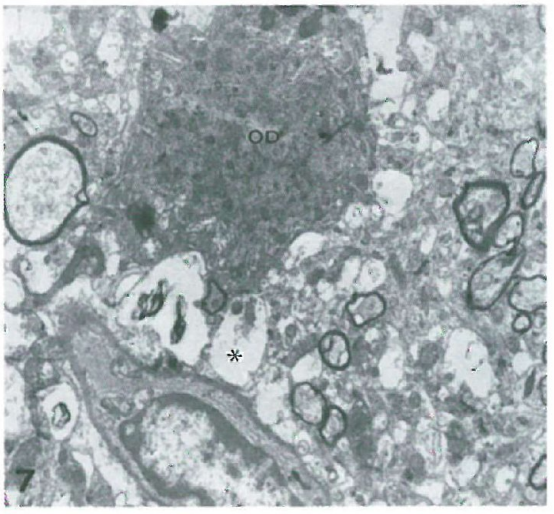

Fig. 7. In the alcohol+moclobemide treated group, cellular and nuclear membrane destruction of a dark oligodendrocyte (OD) that shows densely staining cytoplasm, and has more condensed chromatin in this nucleus. In addition, edema in the neuropil is seen (asterisks). Uranyl acetate and Reynold's lead stain (X9000).

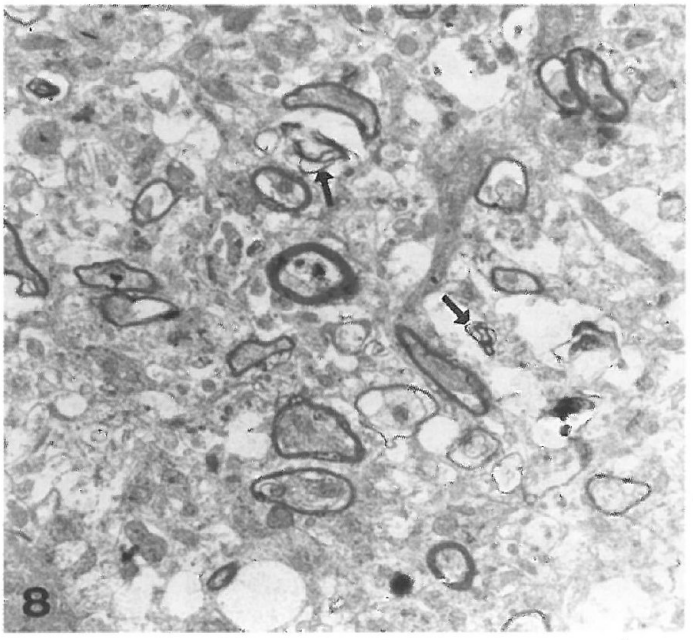

Fig. 8. A transmission electron micrograph of a rat brain from the alcohol+moclobemide group treated shows degenerative myelin structures (arrows). Uranyl acetate and Reynold's lead stain (X7500).

were also observed (Fig. 4).

In the alcohol+moclobemide treated group, most neurons and glial cells of the frontal cortex had increased cytoplasmic degeneration when compared to the alcohol-only treated group (Fig. 5-7). Furthermore, in this group, loss and degeneration in the myelin sheath was significantly greater (Fig. 5,7,8). The lucent wide spaces and degenerative axonal structures were observed in the neuropil (Fig.5-7).

Discussion

This experiment was undertaken to demonstrate the effect of moclobemide on alcoholized rat brain. It was found that the serum ethanol level of the moclobemide+ethanol treated group was significantly higher than that of the ethanol only treated group. It has been suggested that moclobemide inhibits the elimination of ethanol from the body *(Stoecke & Pfefer, 1990)*. Our result has now shown that to be the case - thereby causing an increase in concentration of ethanol in blood serum. However, the mechanism of this effect is still unknown. More studies are needed.

It was also found that myelin degeneration and cellular-nuclear membrane disruption were significantly higher in the alcohol+moclobemide group when compared to the alcohol-only treated group. These results are consistent with the following studies. Cortical morphometric studies in chronic alcohol consumption revealed that neuron loss is most prominent in the frontal cortex, the area suggested to be the most alcohol-sensitive area in the brain (*Harper & Kril, 1985*). Paula-Barbosa et al. (1989) demonstrated that the adult rat cerebellum is particularly sensitive to alcohol effects. Besides the changes in the number and structure of cellular organelles, they observed a progressive degenerative activity, including cell death, in all cerebellar cortical layers, more conspicuous in the granular layer showing a reduction of 30% in the number of granule cells after 18 month of alcohol administration (*Stefanis & Merz, 1990*). In another study, the effects of

ethanol intoxication on the hippocampus was studied in Sprague-Dawley rats and the results confirmed the lethal influence of ethanol on some neurons, and the limited ability of the remnant neurons to compensate for neuronal loss (*Bengoechea & Goncalo,1991*).

Degeneration of phospholipid-rich myelin structure and biological membranes are closely related to the free fatty acid level, that was increased by the free oxygen radicals (*Nordmann et al.,1990; Uysal et al.,1989*). Alcohol abolishes the functions of the antioxidant system leading to an increase in oxygenfree radicals; these in turn may result in neuronal death (*Nordmann et al.,1990; Uysal et al.,1989; Thomson et al.,1988; Gverri & Grisolia,1980; Ikeda & Long,1990*). Recent experimental studies have demonstrated that oxygen free radicals may be important mediators of brain injury and edema, and pharmacological antagonism of oxygen free radicals shows beneficial therapeutic result (*Ikeda & Long,1990*). Since myelin degeneration and cellular-nuclear membrane disruption were greater in the moclobemide+ethanol treated group, this might be due to the moclobemide-induced increase in plasma ethanol level that produced free oxygen radicals.

We also found that the cytoplasmic vacuolar degeneration, dilatation of the endoplasmic reticulum-cisterna, increased lysosomal dense bodies and lipid droplets and destruction of mitochondrial cristae were more severe in the moclobemide–ethanol treated group than in the ethanol-only treated group. These results were probably also due to the increased alcohol intoxication produced by moclobemide.

It was concluded that moclobemide increases the toxic effect of alcohol by reducing its elimination from the body. This is very important for alcohol addicts. An antidepressant drug usage with alcohol might produce more toxic effect on the brain. However, more studies are needed.

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