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IN VIVO ANTIOXIDANT EFFECT OF TROLOX IN RAT BRAIN, IN MODEL OF PROLONGED ETHANOL INTAKE

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ABSTRACT

PURPOSE: to estimate the effect of Trolox on the level of the Oxidative Stress in the brain (cortex, hippocampus and hypothalamus), using rat model of prolonged ethanol intake. MATERIALS AND METHODS: Four groups of male Albino Wistar rats were used as model animals. Two groups lived at standard conditions, and two were used to develop a model of prolonged ethanol intake. Within each model of living conditions, one group received Trolox (SDD 200 mg/kg), and one was provided with saline solution, via gastric tube. After decapitation under anesthesia, the brains were removed and analyzed for the level of the Oxidative stress. The free radicals generation in rat's brain was monitored using the MTT test. RESULTS: Trolox acted as an in vivo antioxidant within the Standard living conditions and the model of prolonged ethanol intake. This in vivo antioxidant effect was associated with the radical scavenging properties of the compound. CONCLUSIONS: Trolox acted as a strong *in vivo* antioxidant in the brain of rats exposed to prolonged ethanol intake.

Key words: Water soluble Vitamin E, Alcoholism, Oxidative Stress.

INTRODUCTION

Profound investigations revealed the impact of the Oxidative Stress (OS) in the brain pathology due to prolonged ethanol intake (1-3). Application of antioxidants helped to diminish the role of this pathway in the development of brain damage due to alcohol abuse. Trolox, the water soluble form of Vitamin E, proved to act as a good scavenger of nytroperoxide and nytroxyl radicals (4). The solubility of this compound in water and ethanol facilitated its resorbtion through the digestive tract. It was observed that if the Cu^{2+}/Cu^{+} redox-cycle is being involved, Trolox acted as prooxidant in the LDL peroxidation. The in vitro oxidative behavior of Trolox is very well investigated, but its in vivo effects on the OS level in different OS models is still not well described. The Aim of this investigation was to estimate the in vivo oxidative behavior of Trolox, in rat model of profound alcohol intake.

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MATERIALS AND METHODS

All chemicals used for treatment of the animals and biochemical analysis, were of a grade pro analysis (SIGMA/ALDRICH). Physiological solution was used to dissolve the Trolox (0.200 g/kgBW in 2 ml). The buffer (PBS of 7.45), the 10% ethanol solutions, and all solutions needed for the biochemical analyses were prepared using distilled water.

Twenty adult male Albino Wistar rats with Body Weight (BW) of 120±20 g. were selected for their affinity to ethanol (5), separated in 4 groups (5 rats each) and left for one week to adapt to the environment. Two groups, receiving via gastric tube either dissolved Trolox (labeled "T"), or Physiological solution only, were each exposed to either Standard living conditions (Groups Std and Std-T) or to prolonged alcohol intake (Groups A and A-T), latter developed for a period of one week and then applied for three weeks.

Standard living conditions (Std): This model consisted of 12/12 hours light/dark cycle, when receiving food and tap water ad libitum. *Prolonged ethanol intake (A)*: The model A consisted of systemic voluntary ethanol intake, with 12/12 hours light/dark cycle, solid food and 10% ethanol solution ad libitum, for three weeks. Tap water was supplied only during the light part of the light/dark cycle. The model A was developed in 7 days, using a sucrose-fade technique (6, 7), combined with additional per os application of 5g/kg ethanol for 5 days, beginning from the second day of the model development.

All animals received standard rodent chow and tap water. They were housed in a quiet room with at 60±1 % humidity, at temperature 20±1 ^{o}C. Prior to decapitation, all animals were deprived of food for one day, while receiving water and ethanol solution, in accordance with the requirements for the biochemical analysis of the Oxidative Stress markers. At the end of the experiment, they were decapitated under anesthesia (ethyl ether). The organs of interest were removed and used for biochemical analysis.

The animals were treated in accordance with the General regulations for Treatment of experimental animals, established by the Ethic Committee in the Medical University of Sofia, in agreement with the Guide to the Care and Use of Experimental Animal Care (Canadian Council on Animal Care guidelines, 1984).

Immediately after decapitation, the brain was removed and washed thoroughly in ice–cold DBM/EDTA solution. The Cortex, Hippocampus and Hypothalamus were extracted, placed in Ice-cold DBM/EDTA-solution, homogenized (2000 turns/min), in ice-cold bath (15%wt in 5 ml PBS pH 7.45), centrifuged at 2500XG for 15 minutes at +4°C, and the supernatanta was extracted for further analysis. After determining the protein content (8,9), the samples were examined for two Oxidative Stress markers: accumulation of Free-radicals (using the MTT method) and Oxidative damage of the tissue (determination of the Malondialdehyde accumulation). Perkin-Elmer 552 UV-VIS Spectrophotometer was used for these determinations.

Assay for free-radicals accumulation: The relative change of the characteristic absorbance of the MTT-formazan (576 nm) formed from MTT in presence of supernatanta, containing 1 mg of proteins was measured for 5 minutes, at 25°C. In one ml cuvette were introduced: supernatanta containing 1 mg proteins, 0.2 ml lyzing solution, 0.2 ml MTT solution, 0.05 ml solution of Xanthine, and PBS (pH 7.45). The blank measurement was performed in absence of supernatanta. The sample and blank measurements were used to calculate the MTT-formazan formed for 1 minute in presence of supernatanta containing 1 mg proteins.

Assay for MDA-determination: The MDA accumulation was estimated as described in (10), but using $FeCl_2$ instead of $FeSO_4$. In a quartz cuvette were introduced supernatanta containing 1 mg proteins, 0.2 ml lyzing solution, 0.02 ml water solution of $FeCl_2$/EDTA, 0.005 ml H_2O_2 (3 mM), and PBS (Ph 7.45). The background was measured in presence of supernatanta containing 1 mg proteins, dissolved in 0.2 ml lyzing solution and PBS (pH 7.45) to 1 ml. The background was removed. The sample and blank measurements were used for calculation of MDA formed for 1 minute in presence of tissue containing 1 mg proteins.

Data management: For each group, the values of the biochemical markers of interest were presented with their corresponding Mean values and Standard deviations. Within each model the living conditions, the effect of Trolox on the markers for the Oxidative Stress and Antioxidant Defense was estimated by presenting the characteristics of a Trolox receiving group ("Trolox") as a percentage of the same characteristic for the group not receiving Trolox ("Control"). The statistical significance among mean values was analyzed using INSTAT program package. After verifying the Standard deviations with Bartlett test, one-way ANOVA with Bonferoni post-test were applied.

RESULTS

The prolonged ethanol intake resulted in an increased free-radicals accumulation **(Fig. 1a)**, along with increased formation of MDA **(Fig. 1b)** in the rat's brain.

This indicated an elevated Oxidative Stress level in the brain of rat exposed to a prolonged ethanol intake compared with this in the brain of rats living at standard conditions.

Application of Trolox resulted in a much weaker elevations of the Oxidative stress markers **(Figueres 1, 2)**.

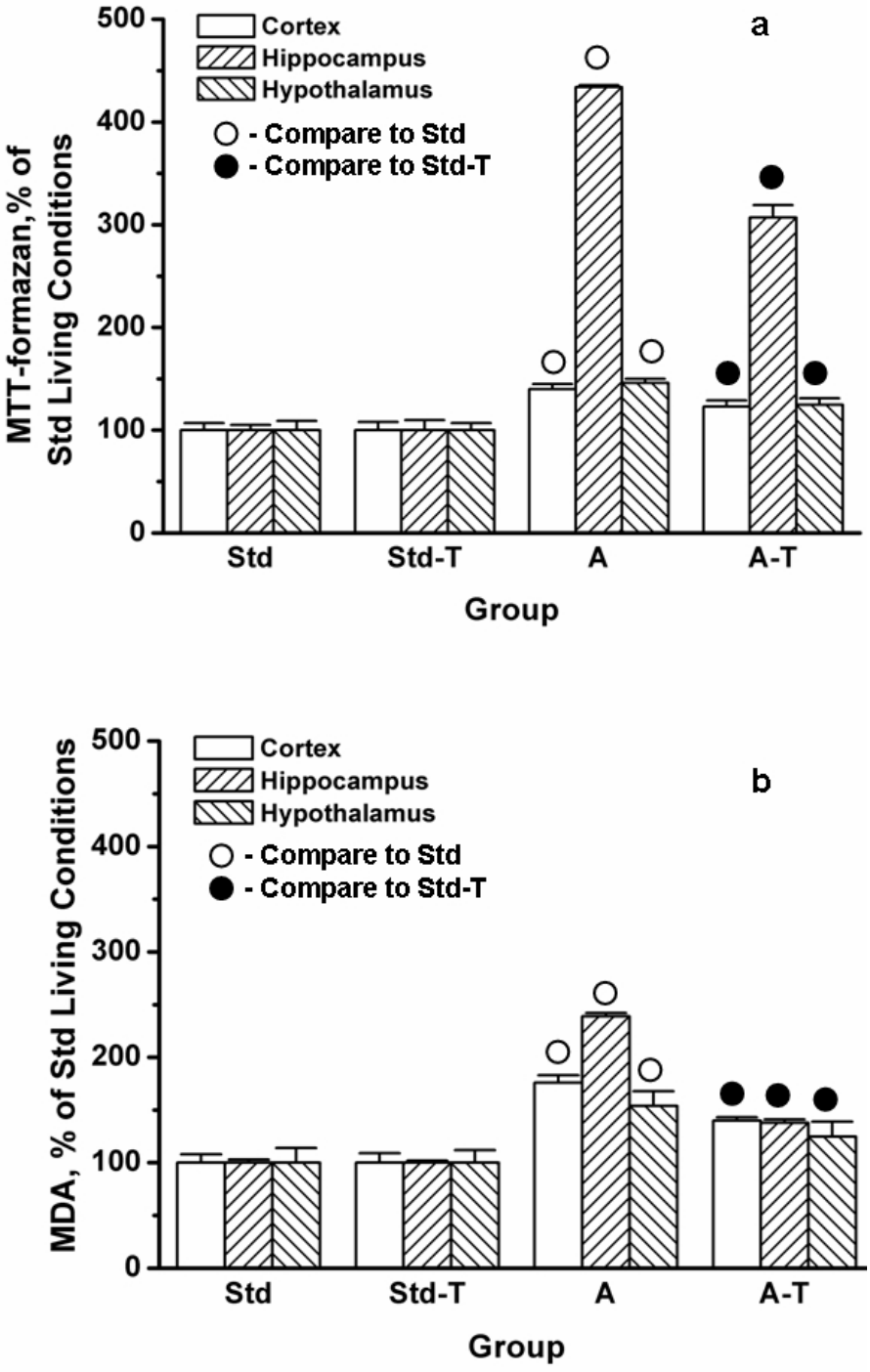

Figure 1. Effect of the living conditions (Standard – Std and Std-T; Prolonged ethanol intake – A and A-T) on the accumulation of free-radicals (a) and Malondialdehyde (b) in the cortex, hippocampus and hypothalamus of rats.

Within the animals living at Standard conditions approximately 20% less free-radicals accumulation **(Fig. 2a)** and a very slight decrease of MDA formation **(Fig. 2b)** were observed. In general, the antioxidant effect of Trolox in the brain of rats living at standard conditions was mild. The free-radicals formation due to prolonged ethanol intake was significantly affected by the in vivo applied Trolox **(Fig. 2a)**. The effect of the compound on the Formation of MDA was similar to this observed for the free-radicals formation **(Fig. 2b)**. It was assumed that the in vivo antioxidant effect of Trolox in the brains of animals exposed to prolonged ethanol intake was stronger than this in the brains of rats living at standard conditions.

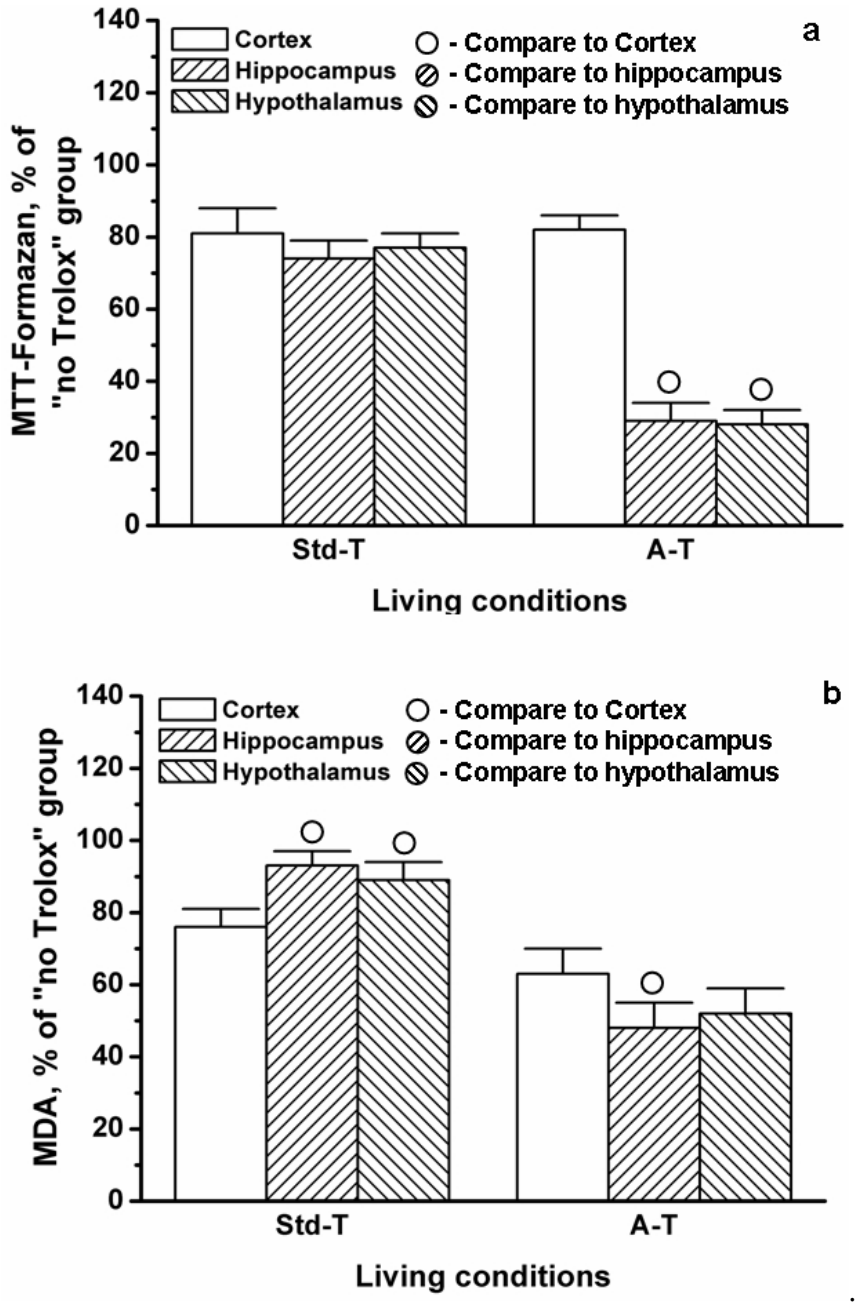

Figure 2. Effect of Trolox on the Free-radicals accumulation (a) and Malondialdehyde formation (b) in the brain cortex, hippocampus and hypothalamus of rat exposed to different living conditions (Standard – Std, and Prolonged ethanol intake – A)

DISCUSSION

The observed increased OS level in the rat brain, due to prolonged ethanol intake compared with standard living conditions was in a good agreement with the literature data (11,12). According (12, 13), ethanol increases the cytozolic NO-synthase (NOS) and induces the microsomal CYP450, this way producing excess of both nitrogen oxide (NO) and superoxide. They interact producing peroxynitrite ($ONOO^-$). Latter is crucial pathogenic factor in development of neurodegenerative diseases (14).

The detected antioxidant effect of Trolox might be related with its in vitro radicals scavenging properties (4). Trolox is as effective as the ascorbic acid in scavenging peroxynitrite and peroxyl radicals, but an order of magnitude less effective scavenger of the hydroxyl radical. It may be presumed that, the

more peroxynitrite and peroxyl radicals formed, the stronger the antioxidant effect of Trolox would be. The fact that in vivo application of Trolox has a positive impact in diminishing the oxidative damage of the brain suggested that most of the free radicals accumulated in the rat's brain due to prolonged ethanol intake may be reactive Nitrogen species by nature.

On the basis of this investigation it was assumed that Trolox might be a promising in vivo antioxidant helping to control over the overproduction of Reactive Nitrogen Species. Non-uniform accumulation of free radicals and MDA were detected within the brain. These variations might be related with specificities of the antioxidant defense within different brain structures.

CONCLUSIONS

Prolonged ethanol intake increased the Oxidative stress in the rat brain cortex, hippocampus and hypothalamus.

It was proposed that the non-uniform accumulation of the OS within the brain might be related with specificities of the antioxidant defense of different brain structures.

Trolox was a promising in vivo antioxidant in the rat brain.

LIST OF ABBREVIATIONS

OS- Oxidative stress
NO- Nitric oxide
ROS- Reactive Oxygen Species
RNS – Reactive nitrogen Species

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