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TROLOX DECREASES THE OXIDATIVE DAMAGE OF THE BLOOD PLASMA IN RAT MODELS OF DIURNAL RHYTHM DISTURBANCE AND PROLONGED ETHANOL INTAKE.

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ABSTRACT
AIM: To estimate the in vivo oxidative behavior of Trolox in the blood plasma in rats models of diurnal rhythm disturbance (D) and prolonged ethanol intake (A), alone and in combination (AD). MATERIALS AND METHODS: Adult male Wistar albino rats were used as model animals. After developing the models of living conditions, part of the animals received a SDD of Trolox (200 mg/kg, per os). Two Oxidative Stress (OS) characteristics were measured in rat blood plasma: the free radicals accumulation (FRA), and the oxidative damage (OD). RESULTS AND DISCUSSION: If living models A, D or AD applied, the OS in the blood plasma increased compared with the normal living conditions (Std). The model AD resulted in highest OS levels in the blood plasma. Application of Trolox substantially decreased the FRA, accompanied by significant decrease of the OD in the blood plasma within models A, D or D. CNCLUSIONS: Trolox proved to be a powerful in vivo antioxidant in the blood plasma of rats exposed to diurnal rhythm disturbance and prolonged ethanol intake, alone or in combination.

Key words: Oxidative stress, insomnia, alcoholism, water soluble Vitamin E

INTRODUCTION

Diurnal rhythm disturbance (1, 2) and Alcohol intake (3,4) result in an oxidative stress (OS) in the body. Vitamin E is a powerful antioxidant, which diminish the oxidative stress due to alcoholism (5) or sleep deprivation (6). Trolox, the water soluble form of α-tokopherol, is well known antioxidant, with radicals-scavenging properties (7). In vitro investigations suggested that the antioxidant effect of the compound depends on its distribution within the biological fluids (8). If Cu^{2+}/Cu^{+} redox-cycle involved, Trolox may exhibit pro-oxidant activity in LDL oxidation. Therefore, the in vivo oxidative behavior of Trolox is not entirely clear. In this work we estimated the in vivo effect of Trolox on some Oxidative Stress markers in the Blood plasma of rats, exposed to such risk factors as prolonged alcohol intake and diurnal rhythm disturbance alone and in combination. The blood plasma is the easiest target for investigating the oxidative stress in vivo (9).

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MATERIALS AND METHODS

All chemicals used were pure pro analisi (SIGMA/ALDRICH). Physiological solution was used to dissolve the Trolox (0.200 g/kgbw in 2 ml). All solutions and buffers in the biochemical analysis were prepared using distilled water.

Forty adult male Albino Wistar rats with Body Weight (BW) of 120±20 g. were selected for their affinity to ethanol (10), separated in 8 groups (5 rats each) and left for one week to adapt to the environment. The animals received standard rodents chow and tub water. They were housed in a quiet room with at 60±1 % humidity, at temperature 20±1 ºC. Two groups, receiving via gastric tube either dissolved Trolox (Trolox" groups), or Physiological solution (Control groups), were

exposed to each of the selected living conditions.

Four living models were applied: Standard living conditions (Std), Prolonged alcohol intake (A), Diurnal Rhythm Disturbance (D), and Prolonged alcohol intake with Diurnal Rhythm Disturbance (AD). The models were developed in one week and applied for three weeks. The "Std" model consisted in 12/12 hours light/dark cycle, when receiving food and tub water ad libitum. The model "A" consisted of voluntary ethanol intake, while 12/12 hours light/dark cycle, with solid food and 10% ethanol solution ad libitum. Tub water was supplied only during the light part of the light/dark cycle within model A. This model was developed in 7 days, using a sucrose-fade technique (11, 12), combined with additional application via gastric tube of 5g/kg ethanol for 5 days, beginning from the second day of the model development. In agreement with literature (13-17), the model "D" was achieved by exposing rats to a 24 hours "day light" illumination with luminescent lamp. Models A and D were simultaneously developed and applied, resulting in model "AD".

Prior 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 (OS) 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 Ethics 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)".

Extraction and storage of organs: Immediately after decapitation, the blood was collected in EDTA-washed vials and the blood plasma was separated via centrifugation (2000HG) for 10 minutes at room temperature and immediately used for biochemical analysis. After determining the proteins content (18, 19), the samples were examined for two Oxidative Stress markers (accumulation of Free-radicals and Malondialdehyde). Perkin-Elmer 552 UV-VIS Spectrophotometer was used for these determinations.

Assay for free-radicals accumulation: The accumulation of free-radicals in presence of supernatanta was estimated by measuring for 5 minutes the formazan formation from MTT, the characteristic absorbance of MTT-formazan being at 576 nm (molar extinction coefficient of 13 $mM^{-1}cm^{-1}$), at 25°C. In one ml cuvette were introduced: blood plasma containing 1 mg proteins, 0.2 ml MTT solution (3mg/ml PBS), 0.05 ml solution of Xanthine (3.5 mM, in water), and PBS (pH 7.45). For the blank measurement, the blood plasma was omitted. After removing the background, the sample and blank measurements were used to calculate the MTT-formazan formed for 1 minute in presence of blood plasma containing 1 mg proteins.

Assay for MDA-determination: The MDA accumulation was estimated as described in (20), but using $FeCl_2$ instead of $FeSO_4$. In a quartz cuvette were introduced blood plasma containing 1 mg proteins, 0.02 ml water solution of $FeCl_2$/EDTA, 0.005 ml H_2O_2 (3 mM), and PBS. The background was measured in presence of blood plasma containing 1 mg proteins and PBS (pH 7.45) to 1 ml. The background was removed. The blood plasma was omitted for the blank measurement. 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 of 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

In **Fig. 1** was observed a significant negative effect of the models A, D, and AD on the OS level in the blood plasma of rats. Within groups not receiving Trolox, the accumulation of free-radicals **(Fig. 1a)** and MDA **(Fig. 1b)** significantly increase due to the living conditions at risk factors, compared with the group living at standard conditions.

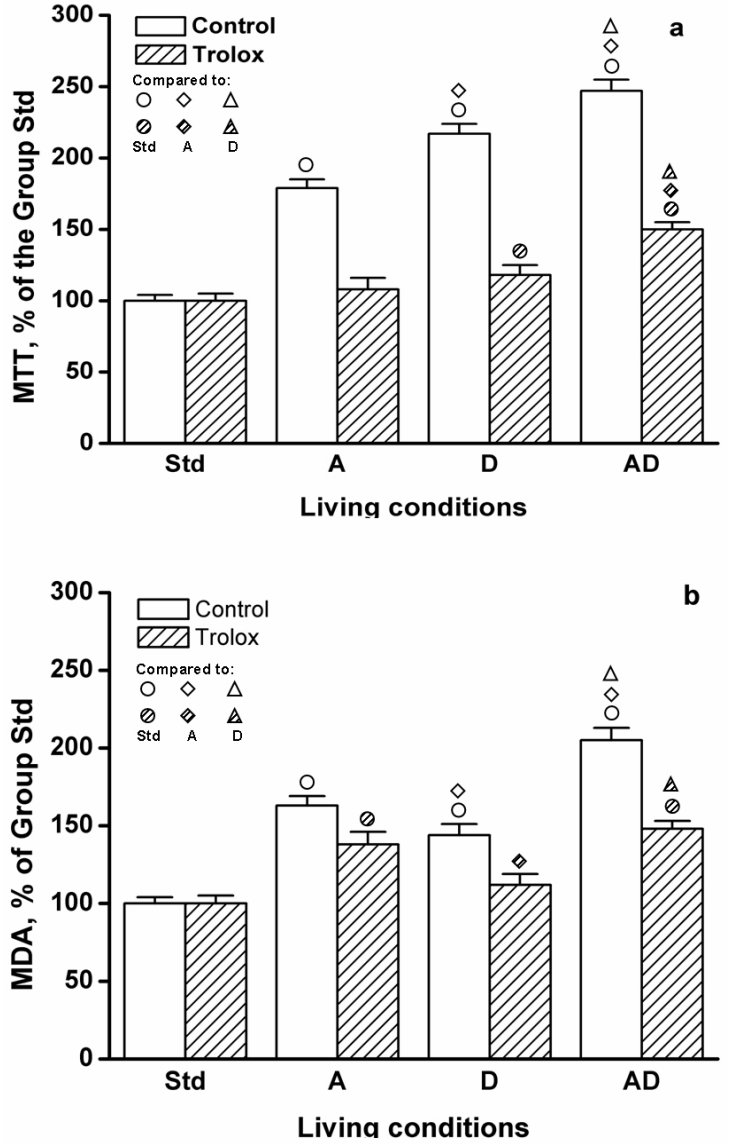

Figure 1. Effect of the living conditions on the free-radicals formation (a) and Oxidative tissue damage (b) in the blood plasma of rats receiving Trolox (Trolox) or physiological solution (Control).

The over-accumulation of free-radicals decreased in the order AD>D>A (Fig.1a), while the amount of MDA detected decreasing in the order AD>A>D (Fig.1b).

Trolox proved to diminish the impact of the risk factors A, D and AD on the OS level in the rat blood plasma (Fig. 1). The extent of this effect was illustrated in Figure 2. Within standard conditions Trolox did not show a statistically significant antioxidant in vivo effect.

If any risk factor (A, D, or AD) applied, then the application of Trolox resulted in significantly lower free-radicals accumulation and MDA formation than these for the group exposed to the same factor but receiving physiological solution only. Stronger effect of Trolox on the FRA than this on the MDA formation was detected.

DISCUSSION

Elevated OS levels due to sleep disturbances and alcoholism have been detected previously (21,22). Our investigation proved these observations, and revealed that the combination of two risk factors as alcohol intake and diurnal rhythm disturbance resulted in higher OS level than any factor alone.

Application of Trolox did not affect the OS markers in the blood plasma of animals living at standard conditions. But if rats were exposed to any of models A, D and AD,

Trolox showed a remarkable in vivo antioxidant effect in the blood plasma. The antioxidant effect of Trolox was much higher on free-radicals accumulation, than on the tissue damage. We believe that this supports its action as scavenger of free-radicals.

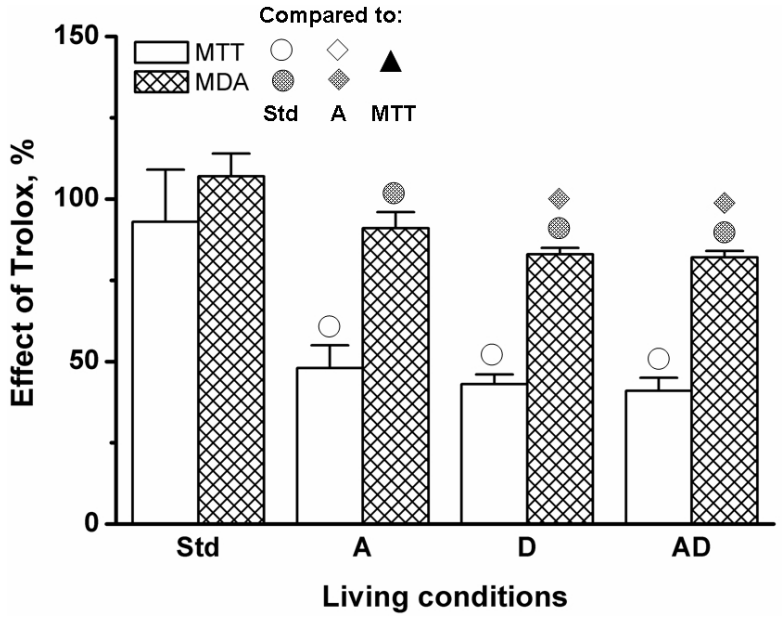

Figure 2. Effect of Trolox (compared with the Control group) on the Free-radicals formation (MTT) and tissue damage (MDA) at each of the models of living conditions applied: Std- standard living conditions, A- prolonged alcohol intake, D- Diurnal Rhythm Disturbance, and AD- Prolonged Alcohol Intake with Diurnal Rhythm Disturbance.

CONCLUSIONS

1. Both diurnal rhythm disturbance and prolonged ethanol intake, alone and in combination, resulted in increased Oxidative Stress in the rat's blood plasma.
2. The simultaneous application of models A and D resulted in higher OS than each model of living conditions alone.
3. Trolox proved to be a powerful in vivo antioxidant in the blood plasma of rats exposed to diurnal rhythm disturbance and prolonged ethanol intake, alone or in combination.

LIST OF ABBREVIATIONS

D- Diurnal Rhythm Disturbance
A- Prolonged Ethanol Intake
FRA- Free radicals accumulation
OS- Oxidative Stress
OTD- Oxidative tissue damage
ROS- Reactive Oxygen Species
RNS- Reactive Nitrogen Species

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