*Bulgarian Chemical Communications, Volume 51, Special Issue A (pp. 167-171) 2019*

# Effect of hypothyroidism and UV irradiation on antioxidant activity via hydrogen donation and electron transfer reactions in rat's blood serum

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Received March 17, 2019; Accepted April 19, 2019

The overall antioxidant defense due to ultraviolet (UV) exposure or hypothyroidism alone has been reported decreased in both humans and animal models. In our previous investigation we found markedly decreased radical scavenging activity (RSA) toward stable 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH$^{\bullet}$) in irradiated hypothyroid rat skin. As the antioxidant defense proceeds via both hydrogen donation and electron transfer reactions, the aim of current study was to investigate how the solar simulated ultraviolet (SSUV) exposure together with hypothyroidism worked on the antioxidant capacity of the rat blood serum via these two pathways.

The antioxidant activity of the hydrogen donors was evaluated using interaction with DPPH$^{\bullet}$ and this of the electron donors toward 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) cation-radical (ABTS$^{\bullet+}$), respectively.

Four groups of male Wistar albino rats, named C (controls), SSUV (irradiated), PTU (hypothyroid), and PTU+SSUV, were used in this study. Drug-induced hypothyroidism was developed by the addition of 0.01% (w/w) 6-n-propyl-2-thiouracil (PTU) for 5 weeks in the *ad libitum* consumed drinking water. Then SSUV and PTU+SSUV groups were irradiated for 7 days, 60 minutes daily.

The results showed that in a hypothyroid state, RSA decreased compared to the controls, by 40 and 20% toward DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, respectively. In SSUV group, RSA(DPPH$^{\bullet}$) did not change, while RSA(ABTS$^{\bullet+}$) decreased by 20%. After SSUV exposure of hypothyroid rats, both RSA(DPPH$^{\bullet}$) and RSA(ABTS$^{\bullet+}$) decreased by almost 30%. The cumulative effect of PTU-induced hypothyroidism and SSUV radiation on RSA(DPPH$^{\bullet}$) was not so strong here in the animal serum, than as the previously observed in their skin. Nevertheless, the diminished activity in hydrogen donation and electron transfer reactions in the blood serum of the hypothyroid animals exposed to SSUV radiation indicated a potential risk of free radicals accumulation in the blood stream, which might trigger oxidative damage.

**Keywords**: Hypothyroidism, UV radiation, SSUV, antioxidant defense, RSA, DPPH$^{\bullet}$, ABTS$^{\bullet+}$

## INTRODUCTION

Thyroid hormones [1-10] and chronic UV radiation [10-13] have a strong impact on oxidative status of the body and can lead independently to oxidative stress (OS). A decrease in overall antioxidant defense due to hypothyroidism [14-18] or UV exposure [11,19-22] alone has been reported in both humans and animal models. Recently, we first reported for a cumulative effect of both chronic UV radiation and 6-n-propyl-2-thiouracil (PTU)-induced hypothyroidism on certain OS markers in the skin [23]. Our data proved for the first time that solar simulated ultraviolet (SSUV) exposure can lead to a higher lipid peroxidation and skin cell damage in the state of hypothyroidism. Moreover, in our previous investigation using an animal model, we found markedly decreased total antioxidant capacity toward stable 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH$^{\bullet}$) in the skin of hypothyroid rats. We have also observed that SSUV exhausted more hydrogen donating antioxidants than the hypothyroidism, while the combination of the two factors resulted in a very strong decrease of the radical scavenging activity (RSA) [24].

As the antioxidant defense proceeds via both hydrogen donation and electron transfer reactions, the aim of current study was to investigate how SSUV exposure together with hypothyroidism worked on the antioxidant capacity of the rat blood serum via these two pathways.

## MATERIALS AND METHODS

All chemicals used in this study were of the highest available grade (Sigma-Aldrich). Bi-distilled water and 96% ethanol were used as solvents for the preparation of the solutions.

### *Animal model*

Twenty four male Wistar albino rats of body weight (BW) 135±5 g were assigned to 4 groups: C (control), SSUV (euthyroid rats exposed to SSUV radiation), PTU (hypothyroid rats) and PTU+SSUV (SSUV treated hypothyroid rats), all housed in transparent standard containers. The animals were kept at room temperature (25±0.5 °C), standard humidity (60±1 %) and a light/dark (12/12 h) cycle. All animals were treated in agreement with the general regulations for treatment of experimental

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animals, established by the Ethics Committee of the Medical University of Sofia (Kenimus), in agreement with EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

After one week of adaptation, hypothyroidism was induced in the PTU and PTU+SSUV groups by administration of 0.01% (w/w) PTU for five weeks in the *ad libitum* consumed drinking water. The BW of the rats was measured on a daily basis. Average weekly BW gain and average daily BW of each animal for the week were calculated for every group. The average daily dose of PTU consumed by the model animals was determined on the basis of the average daily consumption of PTU solution, and was found to be 16±3 mg/kg BW. At the end of the fourth week of the experiment, thyroid hormones were measured for each group to confirm the hypothyroid state. During the final week, euthyroid (SSUV group) and hypothyroid rats (PTU+SSUV group) received ultraviolet radiation, using a SSUV lamp (type "Helios", UV-125W/IR-175W, IBORA, Bulgaria). The lamp combined UV (180 – 400 nm) and IR sources that were adjusted to mimic sunlight. The SSUV source was positioned at a distance of one meter from the animals' cage. The two groups were irradiated for 15 min four times per day (60 min daily) for seven days with periods of 15 min rest between sessions (UV-45 mJ/sm$^2$; IR-63 mJ/sm$^2$). Our UV irradiation model was modified from Erden Inal *et al.* [25] to avoid radiation-inflicted burns and to mimic low-dose daily sunlight.

After the seventh day of SSUV-exposure, blood samples were collected and used to analyze the reactivity in hydrogen donation and electron transfer reactions.

### *Spectrophotometric measurements*

After measuring the amount of proteins in the blood serum using the protocol described in [26], the samples were diluted with distilled water to a concentration of 1 mg/ml proteins. The serums of each group, containing one mg proteins were used for standard DPPH$^\bullet$ and ABTS$^{\bullet+}$ [2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) cation-radical] assays.

All spectrophotometric measurements were performed using "UV-VIS Shimadzu 1601" equipped with standard software package.

The antioxidant activity of the hydrogen donors was evaluated using interaction with DPPH$^\bullet$ and this of the electron donors was observed using their interaction with ABTS$^{\bullet+}$. As strong the radical scavenging effect of a substance toward a stable radical is, as less the intensity of a characteristic signal for a radical will be, compared with the signal in the absence of this substance. RSA (%) by using of the both methods was calculated as follows:

$$RSA = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} * 100,$$

*A* being the measurement for the control, sample or blank specimen in each of the methods. The control measurement permitted to evaluate the absorbance of the characteristic signal of the stable free radical alone in the medium, taking into account the volume of the serum. The blank measurement considers the effect of the serum on the absorption at the characteristic wavelength for the radical in a medium, in the absence of the radical. This way, ($A_{sample}$ - $A_{blank}$) shows the decay of the characteristic absorption due to radical's scavenging alone. As higher RSA, as better the antioxidant activity related with electron transfer is.

### *Assay for RSA toward DPPH$^\bullet$*

Standard solution of DPPH$^\bullet$ was prepared as we described previously [27]. The relative decrease in absorption of the signal at 517 nm (characteristic band for DPPH$^\bullet$) was monitored for 5 min with 10 seconds lag time using the kinetic software of the apparatus. The absorption at 517 nm was recorded every minute. The total volume of a solution in the cuvette was 2 ml. $A_{control}$ was measured in presence of 1.98 ml DPPH$^\bullet$ solution in ethanol and 0.02 ml distilled water. $A_{blank}$ was monitored in 1.98 ml ethanol and 0.02 ml serum, in the absence of DPPH$^\bullet$. The measurement of $A_{sample}$ was performed in the presence of 1.98 ml DPPH$^\bullet$ solution and 0.02 ml serum. Each RSA was calculated five times based on five parallel blank, control and sample measurements. All RSA for an animal group were used to calculate the mean value and standard deviation for this group. These data, along with the number of parallel measurements were used for statistical evaluation of relative differences among RSA of different groups.

### *Assay for RSA toward ABTS$^{+\bullet}$*

ABTS$^{\bullet+}$ solution in acetate buffer of pH =3.8 (named R2) and medium of Na-acetate buffer of pH=5.8 (named R1) were prepared as described [28]. The characteristic band at 660 nm was selected for the measurement of the interaction of the serum with ABTS$^{\bullet+}$, as it did not coincide with serum's characteristic bands. The total volume of the solution used in this method was 1 ml. $A_{sample}$ was measured in the presence of 0.94 ml R1, 0.02 ml R2 and 0.04 ml blood serum. $A_{control}$ was monitored in presence of 0.94 ml R1, 0.02 ml R2 and 0.04 ml distilled water. The blank measurement

was performed in presence of 0.96 ml R1 and 0.04 ml serum. For each RSA calculated, 5 parallel blank, control and sample measurements were used. All RSA of an animal group were used to find the average value and standard deviation of RSA for this group.

***Statistical analysis***

Each group consisted in 5 animals and represented one individual data point for the group. For each animal we performed 5 parallel measurements. Data for the group resulted from 5 independent measurements, each being repeated 5 times. The relative differences among data for two groups of animals were statistically verified using the INSTAT statistical software package. The significance of differences among standard deviations was verified using Bartlett test. One way ANOVA test was further performed, followed by Bonferroni *post-test*. Differences with $p<0.05$ were accepted to be significant.

**RESULTS AND DISCUSSION**

Similar to our previous experiments [10], here in PTU-induced hypothyroid model, we observed again a negative impact of the drastic decreased thyroid hormones on the growth, weight gain and metabolism of the animals (see Fig.1). The results showed that in comparison with the controls, in a hypothyroid state, RSA in the serum decreased by 40 and 20% toward $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively, as presented in Figs. 2 and 3. Evidently, in the blood serum of hypothyroid rats antioxidants with hydrogen donation activity decreased more that these with electron donation activity. In irradiated euthyroid rats (SSUV group), RSA($DPPH^{\bullet}$) did not change (Fig.2), while RSA($ABTS^{\bullet+}$) decreased by 20% (Fig.3). Interestingly, SSUV exposure did not affect the hydrogen donating antioxidant activity, but resulted in a decreased total capacity of the electron donors.

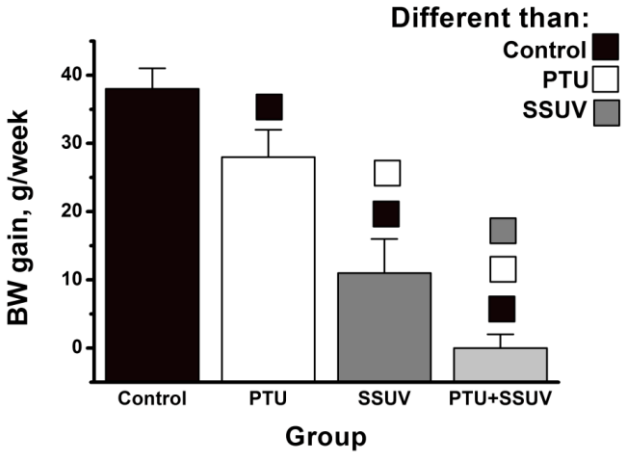


**Fig.1.** Average weekly body weight gain presented as g/week for: Control group, SSUV-exposed group (SSUV); propylthiouracil-induced hypothyroid group (PTU), and propylthiouracil-induced hypothyroid group exposed to SSUV radiation (PTU+SSUV). All data are statistically significant ($p< 0.05$).

After SSUV exposure of the hypothyroid rats, both RSA($DPPH^{\bullet}$) and RSA($ABTS^{\bullet+}$) decreased by almost 30% compared to the controls. It might be assumed that both SSUV and hypothyroidism contribute to the decrease of the antioxidant capacity via both pathways (Fig. 2 and 3). The effects of PTU-induced hypothyroidism and SSUV on RSA ($DPPH^{\bullet}$) in the serum were not as strong as the previously observed in the skin [23].

In addition to our previous findings about reduced antioxidant protection in the skin of hypothyroid rats [24] and other research data showing decreased antioxidant defense measured in the blood of hypothyroid patients [14,29], here we confirm that hypothyroidism can decrease the antioxidant capacity in blood serum via both pathways.

The diminished activity in comparison to negative controls in hydrogen donation and electron transfer reactions in the blood serum of the hypothyroid rats exposed to UV-irradiation was weaker than the exhausted antioxidant capacity in the skin of the same group [24]. The weakened antioxidant defense of the blood serum in SSUV-irradiated hypothyroid rats versus controls indicated a potential risk of free radicals accumulation in the blood stream, which might trigger oxidative damage. It was proposed that treatment with antioxidants with radical scavenging properties would help to diminish this risk.

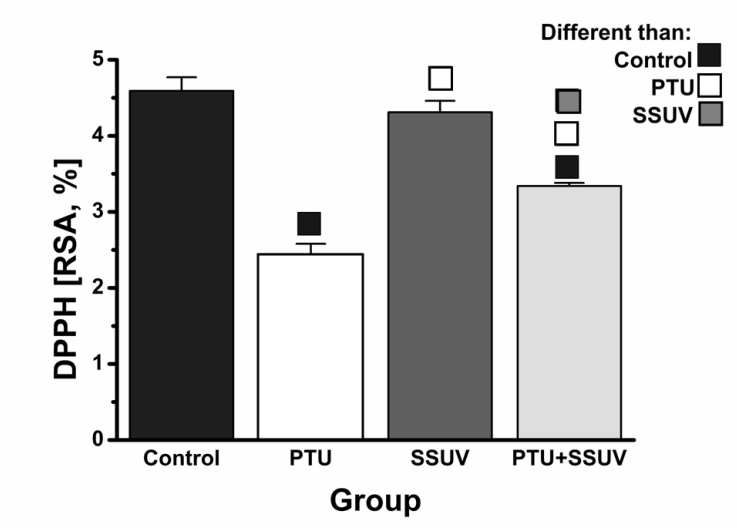


**Fig.2.** Antioxidant capacity of the hydrogen donors evaluated toward DPPH$^{\bullet}$. All data are statistically significant ($p< 0.05$).

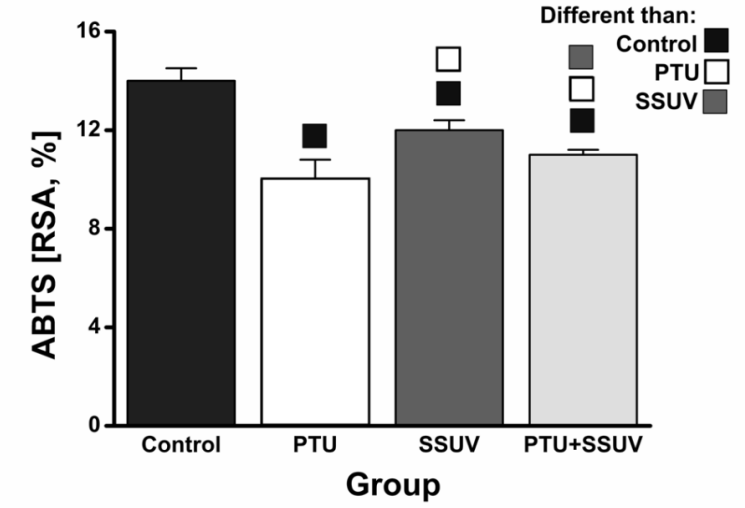


**Fig.3.** Antioxidant capacity of the electron donors observed toward ABTS$^{\bullet+}$. All data show statistical significance ($p< 0.05$).

## CONCLUSIONS

1. In the rat model of hypothyroidism, a diminished hydrogen donation and electron transfer reactions were observed in the blood serum.
2. SSUV treatment of euthyroid rats alone resulted in less decrease of the antioxidant capacities via both pathways, compared with the corresponding decreases in these capacities in hypothyroid state (PTU group).
3. The SSUV-exposure of hypothyroid animals resulted in almost 30% decrease of the hydrogen donation and electron transfer reactions in comparison with the controls, indicating a weakened antioxidant defense of the blood serum.

***Acknowledgement.*** *This work was financially supported by Grant № 73/2013 of the Scientific Board at the Medical University of Sofia, Bulgaria.*

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