



## IN VITRO CHEMILUMINESCENCE INVESTIGATION OF THE ANTIOXIDANT PROPERTIES OF YOHIMBINE

L. Todorov<sup>1</sup>, V. Hadjimitova<sup>2</sup>, M. Traykova<sup>3</sup> and T. Traykov<sup>2,4</sup>

<sup>1</sup>Faculty of Pharmacy, Medical University of Sofia.

<sup>2</sup>Department of Physics and Biophysics, Medical Faculty, Medical University of Sofia,  
2 Zdrave Street, 1431 Sofia, Bulgaria.

<sup>3</sup>Department of Pharmacology and Toxicology, Medical Faculty, Medical University of Sofia, 2  
Zdrave Street, 1431 Sofia, Bulgaria,

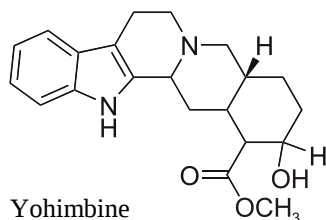
<sup>4</sup>Correspondence, email: ttraykov@mail.bg

### ABSTRACT

The aim of this investigation was to measure the activity of yohimbine as antioxidant and scavenger against ROS. The measurement was performed using a luminol-dependent chemiluminescence (CL) in the presence of *in vitro*-generated  $\cdot\text{O}_2^-$ ,  $\cdot\text{OH}$  and  $\text{OCl}^-$ . We found that the luminol-dependent CL in presence of yohimbine was less intensive than in absence of the drug. The strongest effect was observed for  $\text{OCl}^-$  a little bit weaker for  $\cdot\text{OH}$ . On the contrary, the effect was much lower for the superoxide radical. On the basis of these results it was proposed that yohimbine hydrochloride has some antioxidant activity *in vitro* and might be involved in the control over the oxidative stress.

**Keywords:** yohimbine, chemiluminescence, superoxide, hydroxyl radical, hypochlorous

### INTRODUCTION



The bark of *Pausinystalia yohimbe* is traditionally used in Africa as hallucinogenic agent, aphrodisiac, against fevers and leprosy. Its therapeutic effects have been related mainly with yohimbine. By blocking the  $\alpha$ -2-adrenergic receptors, yohimbine simultaneously suppresses the sympathetic nervous system and increases the norepinephrine in the blood stream. Latter acts as a hormone (adrenergic action), and causes amphetamines-like effects. Yohimbine acts as MAO inhibitor, as well. None of these types of biological activity can explain the effectiveness of yohimbine against fevers. The yohimbine molecule contains both -OH and -NH groups, which might potentiate some antioxidant activity. This antioxidant activity might help to reduce the oxidative damage on the host's tissues, due to inflammatory excitation of

neutrophils. The aim of the present investigation was to estimate *in vitro* the antioxidant properties of yohimbine.

### MATERIALS AND METHODS

All reagents used were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and were of finest grade. The luminol-dependent chemiluminescence (CL) was used for registration of ROS [1,3] with an LKB 1251 luminometer (Bioorbit, Turku, Finland) set at 310 K, and connected with an AT-type computer as described [3,5]. Data collection was performed by MultiUse pro-program version 1.08 (Bioorbit, Turku, Finland). Three types of CL assays were used. The ratio of CL in the presence and absence of the compound investigated was named CL scavenging index (CL-SI) [5] and presented in percent. Three types of CL assays were used.

**Assay I :** Luminol-dependent CL in a system of NaOCl-generated  $\text{ClO}^-$ . The sample contained the following substances in PBS: 0.1 mmol/l luminol, 0.06 mmol/l NaOCl and yohimbine of concentrations between 0.1 and 100  $\mu\text{mol/l}$ , or a buffer for the controls. The chemiluminescence

was registered after addition of NaOCl using the “flash assay” option of the MultiUse program, every 50 milliseconds.

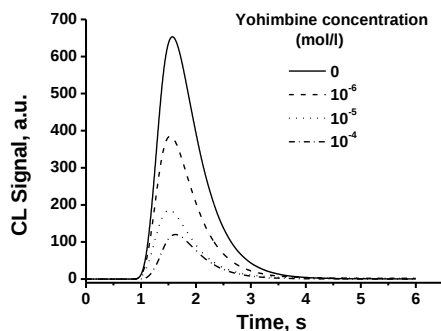
**Assay II:** Luminol-dependent CL in a system of iron-dependent hydroxyl radical formation. Samples (1 ml in PBS solution at pH=7.4) contained 0.1 mmol/l luminol, 0.1 mmol/l  $\text{Fe}^{3+}$  ( $\text{FeCl}_3$ ), 0.1 mmol/l EDTA, 0.1 mmol/l ascorbate, 1 mmol/l  $\text{H}_2\text{O}_2$  and the tested drug at concentrations between 0.1 and 100  $\mu\text{mol/l}$ , or a buffer for the controls. The CL was measured using the “flash assay” option of the MultiUse program, every 50 milliseconds.

**Assay III:** Luminol-dependent CL in a system of xanthine-xanthine oxidase (XO) - generated  $\cdot\text{O}_2^-$ . The sample contained the following substances in 1 ml PBS: 1 mmol/l xanthine, 0.1 mmol/l luminol and the tested drug with concentrations between 0.1 and 100  $\mu\text{mol/l}$ , or a buffer for the controls. The chemiluminescence was registered after addition of 20  $\mu\text{l}$  (100 UI/l) of XO.

Statistics was performed with GraphPad InStat software, version 2.04, USA. Data are expressed as mean  $\pm$  S.D.

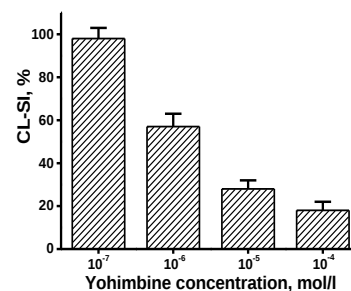
## RESULTS AND DISCUSSION:

The measurements of the luminol-dependent CL in presence of  $\text{OCl}^-$  showed that yohimbine is an antioxidant with strong influence on CL signal within the concentration range  $10^{-6}$ - $10^{-4}$  mol/l. On Fig 1 are shown typical CL kinetic curves for such system at concentrations from 0 (controls) to  $10^{-4}$  mol/l.



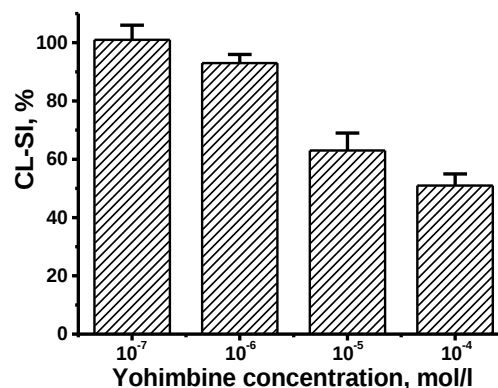
**Fig. 1.** Effect of yohimbine on CL kinetics induced by  $\text{OCl}^-$  generation.

The corresponding CL-SI values are presented in Fig.2. It can be seen that yohimbine have a strong scavenging effect against  $\text{OCl}^-$  even at concentrations of  $10^{-6}$  mol/l (or greater).

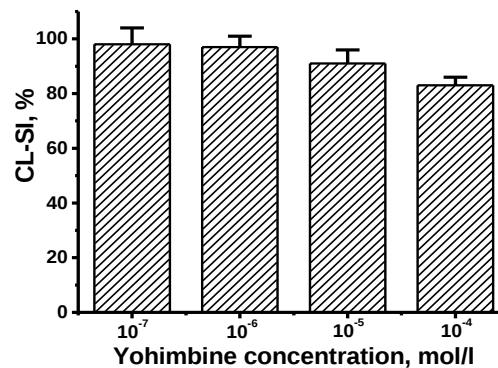


**Fig. 2.** Antioxidant activity of yohimbine for hypochlorous anion ( $\text{OCl}^-$ ).

The effect of yohimbine on CL-SI is weaker for systems containing  $\text{Fe}^{3+}$  - EDTA,  $\text{H}_2\text{O}_2$ , ascorbate - generated  $\cdot\text{OH}$  radicals (Fig. 3).



**Fig. 3.** Antioxidant activity of yohimbine for  $\cdot\text{OH}$  radicals generated in system containing  $\text{Fe}^{3+}$ -EDTA,  $\text{H}_2\text{O}_2$  and ascorbate.



**Fig. 4.** Antioxidant activity of yohimbine for superoxide radicals generated by xanthine – xanthine oxidase reaction.

The weakest antioxidant effect was observed for the superoxide radical (Fig. 4). The decrease of CL-SI was significant for the highest yohimbine concentration of  $10^{-4}$  mol/l and it was only about 10%.

Hypochlorous acid, an important oxidizing agent produced by neutrophils to attack pathogens, is known to cause a tissue damage to the host as well [2]. There are evidences that HOCl may contribute to the inflammatory responses by activating the cells of the immune system, to produce  $\text{TNF}\alpha$ . As endogenous pyrogen,  $\text{TNF}\alpha$  stimulates the heat production and decrease the heat loss, leading to a fever. If the concentration of neutrophil-derived HOCl is reduced by a scavenger molecule, this would diminish the tissue damage of the host, and the  $\text{TNF}\alpha$  production as well. This would help the host to overcome the fever. By the present study we have shown that yohimbine is a strong scavenger for HOCl and this might be one of the possible explanations for the beneficial effect of yohimbine against fever.

## CONCLUSIONS

Yohimbine hydrochloride exhibited a significant *in vitro* scavenging effect against  $\text{OCl}^-$ . It was proposed that yohimbine hydrochloride might be involved therefore in the control over the oxidative stress due to activated neutrophils.

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