Clin. Lab. 2024;70:XXX-XXX
©Copyright

LETTER TO THE EDITOR

Effects of pH on the *in vitro* Antioxidant Activity of Uric Acid

Bruna G. Rossato, Rafael N. Moresco

Department of Clinical and Toxicological Analysis, Center of Health Sciences, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2023.230903)

Correspondence:
Prof. Rafael Noal Moresco
Universidade Federal de Santa Maria
Centro de Ciencias da Saude
Departamento de Analises Clínicas e Toxicologicas
Avenida Roraima 1000, Predio 26, Sala 1401
Camobi, 97105-900, Santa Maria
RS, Brazil
Phone: +55 55 32208941
Fax: +55 55 32208018
Email: rnmoresco@ufsm.br

KEYWORDS

antioxidant, oxidative stress, pH, uric acid

LETTER TO THE EDITOR

Free radicals are highly reactive molecules that promote various toxic effects on human cells [1]. On the other hand, there are molecules with antioxidant action that can protect and repair the damage caused by free radicals on human cells. Uric acid (UA), the end product of the metabolism of purines, is one of these molecules that also has an antioxidant effect in the body [2]. UA can chelate metal ions and transform them into less reactive forms [1] and acts on hypochlorous acid by reducing protein oxidation [3]. Furthermore, UA can eliminate oxidizing agents such as hydroxyl radical, superoxide, and singlet oxygen [4]. These mechanisms prevent neuronal changes associated with diseases such as Alzheimer's [1,5] and Parkinson's [6], which have been associated with low serum levels of UA. The ideal pH for maintaining homeostasis in the body ranges from 7.35 to 7.45 [7,8]. However, some disorders can affect the acid-base balance, causing acidosis or alkalosis [7-9]. As changes in pH can affect various functions in the body, including enzymatic activity, the antioxidant activity of UA may be influenced by variations in pH. Thus, the aim of this study was to evaluate *in vitro* the impact of pH on the antioxidant activity of UA at different concentrations.

UA was prepared at different concentrations (4 mg/dL, 8 mg/dL, and 12 mg/dL) in 0.1 M phosphate-buffered saline (PBS) with pH adjusted to 7.0, 7.4, and 7.8. The assay was performed in triplicate. The pH values aimed to simulate physiological conditions, acidosis, and alkalosis. After the incubations performed at 37°C, the UA concentration was quantified using the commercial kit

Letter to the Editor accepted October 4, 2023

Table 1. Influence of pH on the antioxidant activity of uric acid measured by the ferric reducing ability of plasma (FRAP) assay (µmol/L) in different experimental conditions.

Conditions	pH 7.0	pH 7.4	pH 7.8	p
PBS	139.0 ± 2.0	135.7 ± 3.0	141.0 ± 1.0	0.062
UA 4 mg/dL	172.7 ± 2.0	172.7 ± 4.7	174.0 ± 3.0	0.864
UA 8 mg/dL	206.0 ± 4.5	208.7 ± 1.5	204.7 ± 3.5	0.407
UA 12 mg/dL	227.3 ± 3.0	239.0 ± 1.7	236.3 ± 4.9	0.015

Data are expressed as mean ± standard deviation (SD). PBS - Phosphate-buffered saline, UA - Uric acid.

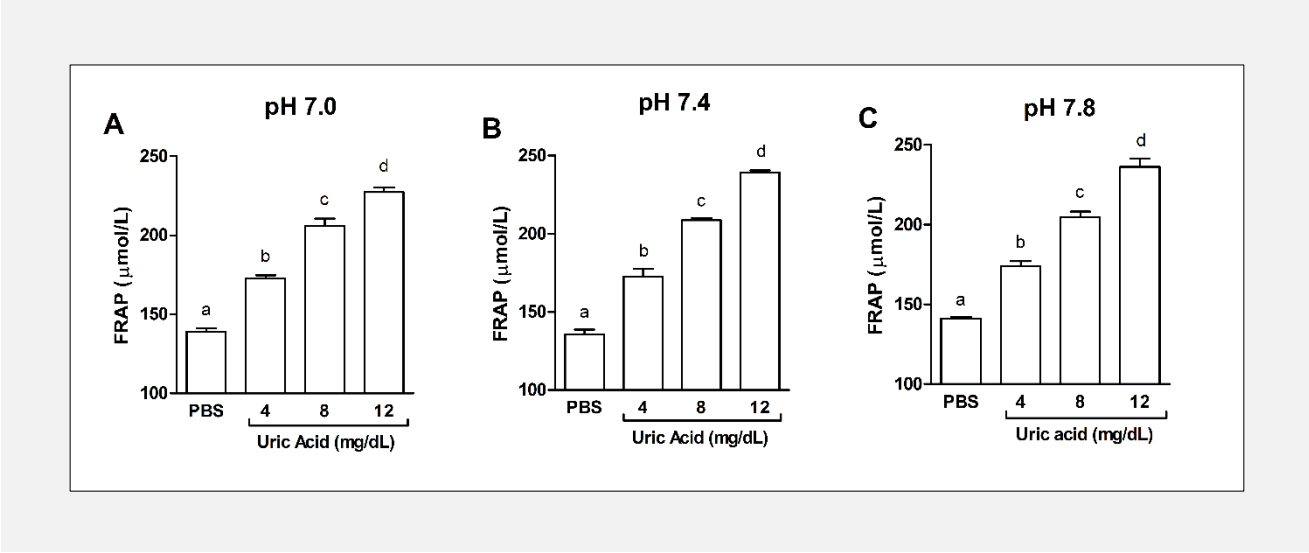

Figure 1. Concentration-dependent effects of pH on the antioxidant activity of uric acid at (A) pH 7.0, (B) pH 7.4, and (C) pH 7.8.

Data are expressed as mean ± standard deviation. Different letters indicate statistical difference between the groups ($p < 0.05$). FRAP - ferric reducing ability of plasma, PBS - Phosphate-buffered saline.

Bioclin® (Quibasa, Belo Horizonte, MG, Brazil). In addition, the ferric reducing ability of plasma (FRAP), an assay used to evaluate antioxidant activity, was evaluated according to Benzie and Strain (1996) [10]. Analyses were performed using the BS 380® automated analyzer (Mindray, Shenzhen, China). Results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and Tukey's post-hoc test were applied to verify differences between groups. $p < 0.05$ was considered statistically significant. Data were analyzed using GraphPad Prism® software version 6.00 for Windows® (La Jolla, CA, USA).

High FRAP values are associated with increased antioxidant activity. Thus, UA showed concentration-dependent antioxidant activity in all tested pH conditions, as shown in Figure 1. Although there is previous evidence that investigated the antioxidant potential of UA in different situations, this is the first time that the impact of the pH of the medium on the antioxidant activity of UA in experimental conditions was studied. This evaluation showed that pH variation did not cause significant changes in the antioxidant action of UA in most conditions tested. However, only at a concentration of 12 mg/dL, associated with severe hyperuricemia, was a statistically significant change in antioxidant activity, as shown in Table 1.

In summary, this *in vitro* investigation demonstrated that UA presents antioxidant activity in a concentration-dependent manner and that pH variations within a range of physiological variations do not influence this activity. A slight variation was found only at the highest concentration of UA, which is not commonly observed in the population. Thus, UA is an important laboratory marker to consider in several scenarios beyond gout investigation.

Source of Fund:
R. N. Moresco received a research productivity scholarship from the National Council for Scientific and Technological Development (CNPq, Brazil, number 313379/2021-1).

Declaration of Interest:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References:

1. Alvarez-Lario B, Macarron-Vicente J. Uric acid and evolution. Rheumatology (Oxford) 2010 Nov;49(11):2010-5. (PMID: 20627967)

2. Ndrepepa G. Uric acid and cardiovascular disease. Clin Chim Acta 2018 Sep;484:150-63. (PMID: 29803897)

3. Carvalho LAC, Truzzi DR, Fallani TS, et al. Urate hydroperoxideoxidizes human peroxiredoxin 1 and peroxiredoxin 2. J Biol Chem 2017 May;292(21):8705-15. (PMID: 28348082)

4. Kutzing MK, Firestein BL. Altered uric acid levels and disease states. J Pharmacol Exp Ther 2008 Jan;324(1):1-7. (PMID: 17890445)

5. Qiao M, Chen C, Liang Y, Luo Y, Wu W. The Influence of Serum Uric Acid Level on Alzheimer's Disease: A Narrative Review. Biomed Res Int 2021 Jun;2021:5525710. (PMID: 34124244)

6. Annanmaki T, Muuronen A, Murros K. Low plasma uric acid level in Parkinson's disease. Mov Disord 2007 Jun;22(8):1133-7. (PMID: 17443703)

7. Ayers P, Dixon C, Mays A. Acid-base disorders: learning the basics. Nutr Clin Pract 2015 Feb;30(1):14-20. (PMID: 25533439)

8. Tucker AM, Johnson TN. Acid-base disorders: A primer for clinicians. Nutr Clin Pract 2022 Oct;37(5):980-9. (PMID: 35752932)

9. Rodriguez-Villar S, Do Vale BM, Fletcher HM. The arterial blood gas algorithm: Proposal of a systematic approach to analysis of acid-base disorders. Rev Esp Anestesiol Reanim (Engl Ed) 2020 Jan;67(1):20-34. (PMID: 31826801)

10. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996 Jul;239(1):70-6. (PMID: 8660627)